$BRAF^{V600E}$ mutation is not associated with central lymph node metastasis in all patients with papillary thyroid cancer: Different histological subtypes and preoperative lymph node status should be taken into account

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Abstract. The association between central lymph node metastasis (LNM) and risk factors, including the presence of the BRAF mutation, $BRAF^{V600E}$, in patients with papillary thyroid cancer (PTC) requires further investigation. A potent risk factor that can indicate LNM in different histological subtypes of PTC and in different preoperative central lymph node statuses also requires further research. A total of 287 patients with PTC who accepted thyroidectomy were included in the present study. Clinicopathological data of these patients were reviewed to examine the risk factors for central LNM through univariate and multivariate analyses. Overall, $BRAF^{V600E}$ in patients with cN0 (subclinical nodal disease) and cN1 (other than cN0) PTC was associated with central LNM. However, multivariate analyses demonstrated that $BRAF^{V600E}$ was not an independent risk factor in patients with cN1 or cN0 PTC. For patients with classical variant PTC (CVPTC), $BRAF^{V600E}$ was independently associated with central LNM. However, on further analysis, the association was only significant in patients with cN0 CVPTC. For patients with follicular variant PTC (FVPTC) or aggressive variant PTC (AVPTC), the $BRAF^{V600E}$ mutation rate was not significantly different between patients with and without central LNM. In conclusion, $BRAF^{V600E}$ was an independent risk factor for central LNM overall in patients with PTC and in patients with CVPTC, particularly in patients with cN0 CVPTC. However, $BRAF^{V600E}$ was not an independent risk factor for patients with FVPTC and AVPTC. Therefore, $BRAF^{V600E}$ provides varied clinical significance in different histological subtypes and preoperative central lymph node status.

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Abbreviations: PTC, papillary thyroid cancer; LNM, lymph node metastasis; LND, lymph node dissection; CVPTC, classical variant PTC; FVPTC, follicular variant PTC; AVPTC, aggressive variant PTC

Key words: papillary thyroid cancer, BRAF mutation, histological subtype, prophylactic dissection, lymph node metastasis

Introduction

Thyroid cancer is the most common endocrine system malignancy and accounts for ~1% of all cancer diagnoses, with an incidence that increases each year (1). Among thyroid malignancies, papillary thyroid cancer (PTC) is the most common histological type, accounting for 80-85% of all thyroid cancer and presenting with a 10-year survival rate of >90% (2). PTC is not a unitary carcinoma; this malignancy includes several sub-histological variants, including classical variant PTC (CVPTC) and follicular variant PTC (FVPTC). Tall cell, diffuse sclerosing and poorly differentiated PTC subtypes are categorized as aggressive variant PTC (AVPTC). Although patients with PTC typically demonstrate an excellent prognosis, cervical lymph node metastasis (LNM) can be identified in 40-90% of cases at the time of the first surgery (3). The most typical LNM site is the central neck compartment (level VI). LNM has been confirmed to be an independent risk factor for regional recurrence (4-6) and decreased survival (7-9). The American Joint Committee on Cancer advocates the surgical removal of suspected lymph nodes detected by preoperative ultrasonography (10). However, a routine central neck lymph node dissection (LND) in patients with subclinical nodal disease (cN0) is controversial. On one hand, LNM occurs in 60% of patients with PTC

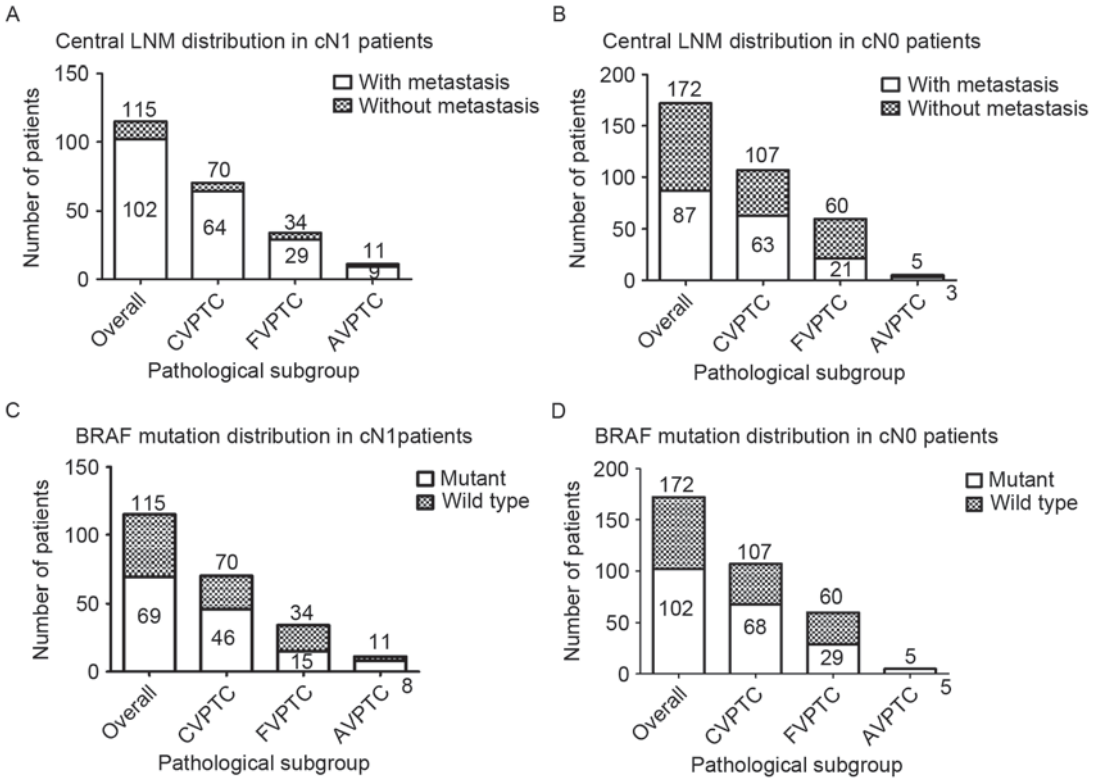

Figure 1. Distribution of central LNM and $BRAF^{V600E}$ in different groups of patients. Central LNM distribution in patients with (A) cN1 and (B) cN0 PTC. The white part of the columns indicates the proportion of patients with central LNM, whereas the shaded part of the columns indicates the proportion of patients without central LNM. The numbers above the columns represent the total number of patients in the particular subgroup. The numbers in the columns represent the number of patients with central LNM. BRAF mutation distribution in patients with (C) cN1 and (D) cN0 PTC. The white part of the columns indicates the proportion of patients with BRAF mutation, whereas the shaded part of the columns indicates the proportion of patients without BRAF mutation. The numbers above the columns represent the total number of patients in the particular subgroup. The numbers in the columns represent the number of patients with BRAF mutation. BRAF, B-Raf proto-oncogene; PTC, papillary thyroid cancer; CVPTC, classical variant PTC; FVPTC, follicular variant PTC; AVPTC, aggressive variant PTC; cN0, subclinical nodal disease; cN1, all other patients (not cN0).

on average (11) and metastasis has been reported in ≤50% of dissected lymph nodes in cN0 patients (12). Prophylactic LND can remove subclinical metastatic lymph nodes, thus avoiding potential recurrence in addition to aiding in disease staging for radioiodine therapy (4,13). On the other hand, previous studies have reported that prophylactic LND in cN0 patients increases the rate of recurrent laryngeal nerve injury and hypoparathyroidism, which would be disadvantageous for patients with PTC (14,15).

The T1799A nucleotide transversion in the B-Raf proto-oncogene (BRAF) gene (NM_004333) causes a V600E amino acid substitution and leads to constitutive activation of the mitogen-activated protein kinase signaling pathway (16). Previous studies have demonstrated that $BRAF^{V600E}$ is the most frequent genetic change in PTC, with prevalence ranging from 27 to 83% (17,18). The association between $BRAF^{V600E}$ and central LNM has been widely investigated. The majority of studies support that $BRAF^{V600E}$ is associated with the presence of LNM and the probability of recurrence (18-20). Certain studies considered that patients with the $BRAF^{V600E}$ mutation should receive LND in the central compartment (18,21,22). However, other studies reported that the correlation between $BRAF^{V600E}$ and tumor aggressiveness, involving LNM, was not evident (23,24). Thus, this divergence in the association between $BRAF^{V600E}$ and more aggressive clinicopathological characteristics deserves further in-depth investigation.

To date, to the best of our knowledge, just one previous study has investigated the clinical significance of $BRAF^{V600E}$ for central LNM based on preoperative central lymph node status and various histological subtypes of PTC (25). Therefore, the present study was designed to separately assess the clinical significance of $BRAF^{V600E}$ in patients with cN0 and cN1 PTC with different histological subtypes.

Patients and methods

Patients. A total of 793 patients accepted thyroidectomy in Department of Surgical Oncology of the First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) between January 2007 and April 2011. These patients were diagnosed with PTC during the final pathological examination. All patients came from the eastern coastal regions in China. Eligibility criteria were as follows: Patients' acceptance of total thyroidectomy plus central LND, absence of a history of neck surgery or radiotherapy on the thyroid, and absence of other types of head and neck cancer. A total of 397 patients were excluded from the present study as they only underwent thyroidectomy, 93 were disqualified for having a previous history of neck surgery or radiotherapy on the thyroid gland, and another 16 were omitted for their history of other types of head and neck cancer. Finally, 287 patients with PTC with total thyroidectomy plus central LND were enrolled.

Table I. Univariate and multivariate analyses of associations between central LNM and clinicopathological characteristics in overall patients with PTC.

Clinicopathological characteristic	Total, n	Univariate analyses			Multivariate analyses	
		Central LNM				
		+, n (%)	-, n (%)	P-value	OR (95% CI)	P-value
Total number	287	189	98			
Age at diagnosis, years						
≥45	129	87 (67.44)	42 (32.56)	0.608		
<45	158	102 (64.56)	56 (35.44)			
Sex						
Female	238	155 (65.13)	83 (34.83)	0.567		
Male	49	34 (69.39)	15 (30.61)			
Pathological tumor size, cm						
>2	86	71 (82.56)	15 (17.44)	0.000	2.621(1.365-5.034)	0.004
≤2	201	118 (58.71)	83 (41.29)			
Underlying Hashimoto's thyroiditis						
Present	112	66 (58.93)	46 (41.07)	0.048	0.652 (0.396-1.099)	0.108
Absent	175	123 (70.29)	52 (29.71)			
Multifocality						
Present	85	63 (74.12)	22 (25.88)	0.055		
Absent	202	126 (62.38)	76 (37.62)			
Extrathyroidal extension						
Present	87	68 (78.16)	19 (21.84)	0.004	1.391 (0.702-2.757)	0.344
Absent	200	121 (60.50)	79 (39.50)			
Thyroid capsular invasion						
Present	109	83 (76.15)	26 (23.85)	0.004	1.477 (0.794-2.747)	0.218
Absent	178	106 (56.08)	72 (73.47)			
BRAF mutation						
Positive	171	125 (73.10)	46 (26.90)	0.002	1.708 (1.004-2.094)	0.048
Negative	116	64 (55.17)	52 (44.83)			

LNM, lymph node metastasis; PTC, papillary thyroid cancer; OR, odds ratio; CI, confidence interval; BRAF, B-Raf proto-oncogene.

Clinicopathological data included patients' age at diagnosis, sex, tumor size, Hashimoto's thyroiditis, multifocality, extrathyroidal extension, thyroid capsular invasion, central LNM and tumor histological subtype. If >1 malignant nodule existed in the thyroid gland, the largest nodule was analyzed. cN0 patients were those patients without preoperative clinical or ultrasonic evidence of central LNM and without suspicious lymph nodes identified during surgery; otherwise, the patients were categorized as cN1 patients. The Institutional Review Board of Wenzhou Medical University approved the present study, and written informed consent was obtained from all patients included.

DNA extraction and BRAF mutation analysis. Genomic DNA was isolated from formalin-fixed paraffin-embedded 7-μm tissue sections using a QIAamp DNA FFPE Tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. BRAF exon 15 was amplified via polymerase chain reaction (PCR) with the following primers: Forward, 5'-TCA TAATGCTTGCTCTGATAGGA-3' and reverse, 5'-GGC CAAAAATTTAATCAGTGGA-3'. A 3-μl sample of the DNA template was added into a 50 μl (total volume) reaction containing 25 μl 2X PCR Reagent (containing 0.1 U/μl Taq polymerase, 500 μM dNTP each, 20 mM Tris-HCl, 100 mM KCl, 3 mM $MgCl_2$), 2 μl of each primer, and 18 μl dH_2O (all Tiangen Biotech Co., Ltd., Beijing, China). The PCR conditions were 94˚C for 3 min, followed by 35 cycles of 94˚C for 30 sec, 55˚C for 30 sec, 72˚C for 1 min and 72˚C for 5 min. The quality of the PCR products was confirmed by 2% agarose gel electrophoresis. Each test was repeated in

Table II. Univariate analyses of associations between central LNM and clinicopathological features in overall patients with cN1 PTC.

Clinicopathological characteristic	Total, n	Central LNM(+), n (%)	Central LNM(-), n (%)	P-value
Total number	115	102	13	
Age at diagnosis, years				
≥45	60	53 (88.33)	7 (11.67)	0.898
<45	55	49 (89.09)	6 (10.90)	
Sex				
Female	90	78 (86.67)	12 (13.33)	0.344[a]
Male	25	24 (96.00)	1 (4.00)	
Pathological tumor size, cm				
>2	42	40 (95.24)	2 (4.76)	0.169[a]
≤2	73	62 (84.93)	11 (15.07)	
Underlying Hashimoto's thyroiditis				
Present	42	37 (88.10)	5 (11.90)	1.000[a]
Absent	73	65 (89.04)	8 (10.96)	
Multifocality				
Present	44	38 (86.36)	6 (13.64)	0.750[a]
Absent	71	64 (90.14)	7 (9.86)	
Extrathyroidal extension				
Present	45	43 (95.56)	2 (4.44)	0.063
Absent	70	59 (84.29)	11 (15.71)	
Thyroid capsular invasion				
Present	47	44 (93.62)	3 (6.38)	0.166
Absent	68	58 (85.29)	10 (14.71)	
BRAF mutation				
Positive	69	65 (94.20)	4 (5.80)	0.022
Negative	46	37 (80.43)	9 (19.57)	

[a]P-value using χ^2 test continuity correction. LNM, lymph node metastasis; PTC, papillary thyroid cancer; BRAF, B-Raf proto-oncogene; cN1, all other patients (not cN0).

triplicate, with dH_2O used as the negative control. The PCR products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, USA) on an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) to identify the mutation.

Statistical analysis. Statistical analysis was performed using IBM SPSS software (version 19.0; IBM Corp., Armonk, NY, USA). A univariate analysis was conducted to reveal the risk factors associated with central LNM in different histological subtypes of PTC. Factors that demonstrated significant differences were further tested through multivariate analysis. Data were described as the number of cases for categorical variables. Confrontations between different groups were performed using a χ^2 test. When the sample size was <40 or with a theoretical frequency of <1, Fisher's exact test was used instead. For continuous variables, data were described as the mean ± standard deviation. A multivariate analysis was performed using binary logistic regression. The results were presented as odds ratios (ORs) with 95% confidence intervals (CIs). P<0.05 was considered to indicate a statistically significant difference.

Results

Patient clinicopathological characteristics. A total of 287 patients with PTC were included in the present study. Patient ages ranged between 9 and 77 years, with a mean of 43.82±11.96 years. The female-to-male ratio was 4.86 (49 males vs. 238 females). A total of 1,985 lymph nodes from the central compartment were dissected, among which 837 were identified as cancer metastases by pathologists (2.9 metastatic lymph nodes to 6.9 total lymph nodes dissected from each patient on average). Central LNM was identified in 189 patients (65.85%), and underlying Hashimoto's thyroiditis was identified in 112 patients (39.02%). Other characteristics such as multifocality, extrathyroidal extension and thyroid capsular invasion were identified in 85 (29.62%), 87 (30.31%) and 109 (37.98%) patients, respectively. Among the patients,

Table III. Univariate and multivariate analyses of associations between central LNM and clinicopathological features in overall patients with cN0 PTC.

Clinicopathological characteristic	Total, n	Univariate analyses: Central LNM +, n (%)	Univariate analyses: Central LNM -, n (%)	Univariate analyses: P-value	Multivariate analyses: OR (95% CI)	Multivariate analyses: P-value
Total number	172	87	85			
Age at diagnosis, years						
≥45	69	34 (49.28)	35 (50.72)	0.779		
<45	103	53 (51.46)	50 (48.54)			
Sex						
Female	148	77 (52.03)	71 (47.97)	0.346		
Male	24	10 (41.67)	14 (58.33)			
Pathological tumor size, cm						
>2	44	31 (70.45)	13 (29.55)	0.002	2.633 (1.189-5.832)	0.017
≤2	128	56 (43.75)	72 (56.25)			
Underlying Hashimoto's thyroiditis						
Present	70	29 (41.43)	41 (58.57)	0.047	0.559 (0.288-1.086)	0.086
Absent	102	58 (56.86)	44 (43.14)			
Multifocality						
Present	41	25 (60.98)	16 (39.02)	0.127		
Absent	131	62 (47.33)	69 (52.67)			
Extrathyroidal extension						
Present	42	25 (59.52)	17 (40.48)	0.182		
Absent	130	62 (47.69)	68 (52.31)			
Thyroid capsular invasion						
Present	62	39 (62.90)	23 (37.10)	0.015	1.692 (0.850-3.370)	0.134
Absent	110	48 (43.64)	62 (56.36)			
BRAF mutation						
Positive	102	60 (58.82)	42 (41.18)	0.009	1.882 (0.976-3.629)	0.059
Negative	70	27 (38.57)	43 (61.43)			

LNM, lymph node metastasis; PTC, papillary thyroid cancer; OR, odds ratio; CI, confidence interval; BRAF, B-Raf proto-oncogene; cN0, subclinical nodal disease.

172 (59.93%) were patients with cN0 PTC, whereas the other 115 (40.07%) were patients with cN1 PTC. According to the histological subtype classification, 177 (61.67%) and 94 (32.75%) patients were diagnosed with CVPTC and FVPTC, respectively. The remaining 16 patients (5.57%) were grouped under AVPTC, which included tall cell, diffuse sclerosing and poorly differentiated PTC subtypes.

Distribution of central LNM and BRAF^{V600E}. Among the patients with cN0 PTC, LNM could be identified in 63 patients (58.88%) in the CVPTC group, 21 patients (35.00%) in the FVPTC group and 3 patients (60.00%) in the AVPTC group; the proportion in the cN1 group was 64 (91.43%), 29 (85.29%) and 9 (81.82%) patients in the CVPTC, FVPTC and AVPTC groups, respectively (Fig. 1A and B). Similarly, among all patients with cN0 PTC, BRAF^{V600E} was detected in 68 patients (63.55%) in the CVPTC group, 29 patients (48.33%) in the FVPTC group and all 5 patients (100.00%) in the AVPTC group; the proportion in the cN1 group was 46 (65.71%), 15 (44.12%) and 8 (72.72%) patients in the CVPTC, FVPTC, and AVPTC groups, respectively (Fig. 1C and D).

Univariate and multivariate analyses in all patients with PTC. Among all patients with PTC (Table I), BRAF^{V600E} was associated with a higher rate of central LNM (73.10 vs. 55.17%; P=0.002). Underlying Hashimoto's thyroiditis was negatively correlated with central LNM (58.93 vs. 70.29%, P=0.048). In addition, central LNM occurred more frequently in patients with larger tumor size, extrathyroidal extension, thyroid capsular invasion and BRAF^{V600E} (82.56 vs. 58.71%,

Table IV. Univariate and multivariate analyses of associations between central LNM and clinicopathological features in patients with CVPTC.

Clinicopathological characteristic	Total, n	Univariate analyses: Central LNM +, n (%)	Univariate analyses: Central LNM -, n (%)	Univariate analyses: P-value	Multivariate analyses: OR (95% CI)	Multivariate analyses: P-value
Total number	177	127	50			
Age at diagnosis, years						
≥45	75	56 (74.67)	19 (25.33)	0.460		
<45	102	71 (69.61)	31 (3.39)			
Sex						
Female	148	103 (69.59)	45 (30.41)	0.150		
Male	29	24 (82.76)	5 (17.24)			
Pathological tumor size, cm						
>2	55	48 (87.27)	7 (12.73)	0.002	2.656 (1.049-6.724)	0.039
≤2	122	79 (64.75)	43 (35.25)			
Underlying Hashimoto's thyroiditis						
Present	74	45 (60.81)	29 (39.19)	0.006	0.410 (0.200-0.841)	0.015
Absent	103	82 (79.61)	21 (20.39)			
Multifocality						
Present	50	41 (82.00)	9 (18.00)	0.057		
Absent	127	86 (67.72)	41 (32.28)			
Extrathyroidal extension						
Present	50	43 (86.00)	7 (14.00)	0.008	1.358 (0.470-3.926)	0.572
Absent	127	84 (66.14)	43 (33.86)			
Thyroid capsular invasion						
Present	58	51 (87.93)	7 (12.07)	0.001	2.941 (1.054-8.202)	0.039
Absent	119	76 (63.87)	43 (36.13)			
BRAF mutation						
Positive	114	91 (79.82)	23 (20.18)	0.001	2.243 (1.080-4.658)	0.030
Negative	63	36 (57.14)	27 (42.86)			

LNM, lymph node metastasis; CVPTC, classical variant papillary thyroid cancer; OR, odds ratio; CI, confidence interval; BRAF, B-Raf proto-oncogene.

P<0.001; 78.16 vs. 60.50%, P=0.004; and 76.15 vs. 56.08%, P=0.004, respectively). These significantly different factors were then analyzed by multivariate logistic regression. The results demonstrated that $BRAF^{V600E}$ was independently associated with central LNM with an OR value of 1.708 (95% CI, 1.004-2.094; P=0.048). In addition, large tumor size was an independent risk factor for central LNM with an OR value of 2.621 (95% CI, 1.365-5.034; P=0.004).

Among all patients with cN1 PTC (Table II), $BRAF^{V600E}$ was the only factor correlated with central LNM (94.20 vs. 80.43%; P=0.022). Only 13 patients did not exhibit central LNM in this cohort. Central LNM had a higher prevalence in patients with large tumor size (95.24 vs. 84.93%; P=0.169), extrathyroidal extension (95.56 vs. 84.29%; P=0.063) and thyroid capsular invasion (93.62 vs. 85.29%, P=0.166); however, these results were not statistically significant.

Among all patients with cN0 PTC (Table III), central LNM was identified in 58.82% of $BRAF^{V600E}$-positive patients compared with 38.57% of $BRAF^{V600E}$-negative patients (P=0.009). Additionally, a higher rate of central LNM was revealed in patients with larger tumor size and thyroid capsular invasion (70.45 vs. 43.75%, P=0.002; and 62.90 vs. 43.64%, P=0.015, respectively). Underlying Hashimoto's thyroiditis was negatively correlated with central LNM (41.43 vs. 56.86%, P=0.047). These four significantly different factors were further examined using multivariate analysis. Large tumor size was the only independent risk factor for central LNM with an OR value of 2.633 (95% CI, 1.189-5.832; P=0.017). However, $BRAF^{V600E}$ was not associated with central LNM in the multivariate analysis (P=0.059), which was inconsistent with the overall result for the patients with PTC in the present study.

Table V. Univariate analyses of associations between central LNM and clinicopathological features in patients with cN1 CVPTC.

Clinicopathological characteristic	Total, n	Central LNM (+), n (%)	Central LNM (-), n (%)	P-value
Total number	70	64	6	
Age at diagnosis, years				
≥45	37	35 (94.59)	2 (5.41)	0.411[a]
<45	33	29 (87.88)	4 (12.12)	
Sex				
Female	53	48 (90.67)	5 (9.43)	1.000[a]
Male	17	16 (94.12)	1 (5.88)	
Pathological tumor size, cm				
>2	27	27 (100.00)	0 (0.00)	0.075[a]
≤2	43	37 (86.05)	6 (13.95)	
Underlying Hashimoto's thyroiditis				
Present	25	22 (88.00)	3 (12.00)	0.659[a]
Absent	45	42 (93.33)	3 (6.67)	
Multifocality				
Present	26	24 (92.31)	2 (7.69)	1.000[a]
Absent	44	40 (90.91)	4 (9.09)	
Extrathyroidal extension				
Present	27	27 (100.00)	0 (0.00)	0.075[a]
Absent	43	37 (86.05)	6 (13.95)	
Thyroid capsular invasion				
Present	29	29 (100.00)	0 (0.00)	0.038[a]
Absent	41	35 (85.37)	6 (14.63)	
BRAF mutation				
Positive	46	44 (95.65)	2 (4.35)	0.171[a]
Negative	24	20 (83.33)	4 (16.67)	

[a]P-value using Fisher's exact test. LNM, lymph node metastasis; CVPTC, classical variant papillary thyroid cancer; BRAF, B-Raf proto-oncogene; cN1, all other patients (not cN0).

Univariate and multivariate analyses of patients with CVPTC. A total of 177 patients were diagnosed with CVPTC in the present study; 70 (39.55%) of these patients were cN1, and 107 (60.45%) were cN0. Tumor size >2 cm (87.27 vs. 64.75%; P=0.002), underlying Hashimoto's thyroiditis (60.81 vs. 79.61%; P=0.006), extrathyroidal extension (86.00 vs. 66.14%; P=0008), thyroid capsular invasion (87.93 vs. 63.87%; P=0.001) and $BRAF^{V600E}$ (79.82 vs. 57.14%; P=0.001) were all correlated with central LNM in the univariate analysis (Table IV). The aforementioned factors were then included in the multivariate analysis. Tumor size >2 cm (OR, 2.656; 95% CI, 1.049-6.724; P=0.039), underlying Hashimoto's thyroiditis (OR, 0.410; 95% CI, 0.200-0.841; P=0.015) and thyroid capsular invasion (OR, 2.941; 95% CI, 1.054-8.202; P=0.039) were all independently associated with central LNM. Notably, $BRAF^{V600E}$ was independently associated with central LNM in patients with CVPTC (OR, 2.243; 95% CI, 1.080-4.658; P=0.030).

Similar analyses were performed on 70 patients with cN1 CVPTC (Table V). The results revealed that only thyroid capsular invasion was associated with central LNM (100.00 vs. 85.37%; P=0.038). Other factors, including $BRAF^{V600E}$ (95.65 vs. 83.33%, P=0.171) were not statistically different between the central LNM-positive and -negative groups.

Among the 107 patients with cN0 CVPTC (Table VI), central LNM was correlated with $BRAF^{V600E}$ (69.12 vs. 41.03%; P=0.004), tumor size >2 cm (75.00 vs. 53.16%; P=0.044) and thyroid capsular invasion (75.86 vs. 52.56%; P=0.029). Underlying Hashimoto's thyroiditis was negatively associated with central LNM (46.94 vs. 68.97%, P=0.021). When these factors were included in the multivariate analysis, $BRAF^{V600E}$ was identified to be an independent risk factor for central LNM (P=0.032), with an OR value of 2.586 (95% CI, 1.087-6.151), and underlying Hashimoto's thyroiditis was an independent negative factor in this cohort (P=0.042), with an OR value of 0.411 (95% CI, 0.174-0.970).

Univariate and multivariate analyses of patients with FVPTC. The aforementioned factors were analyzed in a total of 94 patients with FVPTC (Table VII), which comprised 34 patients with cN1 FVPTC (Table VIII) and 60 patients with cN0 FVPTC (Table IX). However, central LNM was

Table VI. Univariate and multivariate analyses of associations between central LNM and clinicopathological features in patients with cN0 CVPTC.

Clinicopathological characteristic	Total, n	Univariate analyses: Central LNM +, n (%)	Univariate analyses: Central LNM -, n (%)	Univariate analyses: P-value	Multivariate analyses: OR (95% CI)	Multivariate analyses: P-value
Total number	107	63	44			
Age at diagnosis, years						
≥45	38	21 (55.26)	17 (44.74)	0.573		
<45	69	42 (60.87)	27 (39.13)			
Sex						
Female	95	55 (57.89)	40 (42.11)	0.787[a]		
Male	12	8 (66.67)	4 (33.33)			
Pathological tumor size, cm						
>2	28	21 (75.00)	7 (25.00)	0.044	2.261 (0.790-6.468)	0.128
≤2	79	42 (53.16)	37 (46.84)			
Underlying Hashimoto's thyroiditis						
Present	49	23 (46.94)	26 (53.06)	0.021	0.411 (0.174-0.970)	0.042
Absent	58	40 (68.97)	18 (31.03)			
Multifocality						
Present	24	17 (70.83)	7 (29.17)	0.177		
Absent	83	46 (55.42)	37 (44.58)			
Extrathyroidal extension						
Present	23	16 (69.57)	7 (30.43)	0.240		
Absent	84	47 (55.95)	37 (44.05)			
Thyroid capsular invasion						
Present	29	22 (75.86)	7 (24.14)	0.029	2.512 (0.885-7.135)	0.084
Absent	78	41 (52.56)	37 (47.44)			
BRAF mutation						
Positive	68	47 (69.12)	21 (30.88)	0.004	2.586 (1.087-6.151)	0.032
Negative	39	16 (41.03)	23 (58.97)			

[a]P-value using χ^2 test continuity correction. LNM, lymph node metastasis; CVPTC, classical variant papillary thyroid cancer; OR, odds ratio; CI, confidence interval; BRAF, B-Raf proto-oncogene; cN0, subclinical nodal disease.

only associated with thyroid capsular invasion in the patients with cN0 FVPTC (P=0.015). None of the other factors were associated with central LNM regardless of preoperative lymph node status. Central LNM rate was not significantly different between $BRAF^{V600E}$-positive and -negative patients (54.55 vs. 52.00%, P=0.805 in all patients with FVPTC; 93.33 vs. 78.95%, P=0.355 in cN1 patients; 34.48 vs. 35.48%, P=0.935 in cN0 patients).

Univariate and multivariate analyses of patients with AVPTC. Given that the patient number in this cohort was limited to 12 and 4 patients in the central LNM-positive and -negative groups, respectively, the risk factors were analyzed in all patients with AVPTC together (Table X) instead of analyzing them separately by preoperative lymph node status. Central LNM rate demonstrated a higher trend in patients with extrathyroidal extension, thyroid capsular invasion (80.00 vs. 72.73% for the two factors) and $BRAF^{V600E}$ (76.92 vs. 66.67%). However, none of these factors were significantly different.

Discussion

Risk factors associated with central LNM in patients with PTC have been widely studied; tumor size, extrathyroidal extension, thyroid capsular invasion, underlying Hashimoto's thyroiditis and $BRAF^{V600E}$ have been reported to be associated with LNM (26-28). $BRAF^{V600E}$ has been demonstrated to be a good risk factor for cervical LNM and was significantly correlated with recurrence (18,19). Nevertheless, certain studies have revealed no correlation between $BRAF^{V600E}$ and cervical LNM (29,30).

Table VII. Univariate analyses of associations between central LNM and clinicopathological characteristics in patients with FVPTC.

Clinicopathological characteristic	Total, n	Central LNM (+), n (%)	Central LNM (-), n (%)	P-value
Total number	94	50	44	
Age at diagnosis, years				
≥45	49	27 (55.10)	22 (44.90)	
<45	45	23 (51.11)	22 (48.89)	0.689
Sex				
Female	78	42 (53.85)	36 (46.15)	0.779
Male	16	8 (50.00)	8 (50.18)	
Pathological tumor size, cm				
>2	24	16 (66.67)	8 (33.33)	0.125
≤2	70	34 (48.57)	36 (51.43)	
Underlying Hashimoto's thyroiditis				
Present	31	16 (51.61)	15 (48.39)	0.83
Absent	63	34 (53.97)	29 (46.03)	
Multifocality				
Present	28	17 (60.71)	11 (39.29)	0.341
Absent	66	33 (50.00)	33 (50.00)	
Extrathyroidal extension				
Present	32	21 (65.63)	11 (34.37)	0.083
Absent	62	29 (46.77)	33 (53.23)	
Thyroid capsular invasion				
Present	46	28 (60.87)	18 (39.13)	0.144
Absent	48	22 (45.83)	26 (54.17)	
BRAF mutation				
Positive	44	24 (54.55)	20 (45.45)	0.805
Negative	50	26 (52.00)	24 (48.00)	

LNM, lymph node metastasis; FVPTC, follicular variant papillary thyroid cancer; BRAF, B-Raf proto-oncogene.

PTC is composed of several distinct histological subtypes, including CVPTC, FVPTC and AVPTC; AVPTC includes tall cell PTC, diffuse sclerosing PTC and poorly differentiated PTC, which all exhibit more aggressive biological behavior (19). In the present study, 287 patients with PTC, comprising 177 patients with CVPTC, 94 patients with FVPTC and 16 patients with AVPTC, were analyzed. Multivariate regression analysis revealed that $BRAF^{V600E}$ was independently associated with central LNM in the overall PTC group, which is in accordance with previous reports (18,19). However, similar results cannot be drawn from FVPTC or AVPTC, as the BRAF mutation rate was not significantly different between these two PTC subtypes. Importantly, $BRAF^{V600E}$ in CVPTC, which is the most common subtype, was a potent independent indicator for central LNM in addition to the overall PTC group. FVPTC presented some similarities with follicular thyroid carcinoma, and $BRAF^{V600E}$ exhibited a relatively low prevalence in this PTC subtype (31). The results from the present study are in agreement with studies by Walts *et al* (32) and Li *et al* (33), which stated that $BRAF^{V600E}$ was not associated with LNM in FVPTC. The difference in $BRAF^{V600E}$ mutation rate was not significant in patients with AVPTC who had a high incidence of $BRAF^{V600E}$ between LNM-positive (83.33%) and -negative (75.00%) groups. The significance of the association between $BRAF^{V600E}$ and LNM in AVPTC may be concealed by such a high rate of $BRAF^{V600E}$. As a consequence, BRAF mutation may perform differently in diverse subtypes. Thus, the divergence in the association between BRAF mutation and central LNM in previous studies may be a result of the different histological subtypes of PTC.

Although previous studies have demonstrated that cervical LNM is associated with local recurrence and even disease-specific survival in patients with PTC (7,34), prophylactic central LND in patients with cN0 PTC remains controversial in thyroid cancer surgery. In the systematic review by Mulla and Schulte (35), cancerous lymph nodes were detected in 46.15% of 1,946 patients with cN0 PTC. Therefore, Mulla and Schulte (35) advocated for prophylactic central LND to be performed on patients with PTC. By contrast, a previous study by Conzo *et al* (15) of 752 patients with cN0 PTC revealed a greater rate of surgical complications, including permanent hypoparathyroidism (3.6 vs. 1%;

Table VIII. Univariate analyses of associations between central LNM and clinicopathological characteristics in patients with cN1 FVPTC.

Clinicopathological characteristic	Total, n	Central LNM (+), n (%)	Central LNM (-), n (%)	P-value
Total number	34	29	5	
Age at diagnosis, years				
≥45	20	16 (80.00)	4 (20.00)	
<45	14	13 (92.86)	1 (7.14)	0.298[a]
Sex				
Female	28	23 (82.14)	5 (17.86)	0.559[a]
Male	6	6 (100.00)	0 (0.00)	
Pathologic tumor size, cm				
≤2	24	21 (87.50)	3 (12.50)	0.618[a]
>2	10	8 (80.00)	2 (20.00)	
Underlying Hashimoto's thyroiditis				
Present	12	11 (91.67)	1 (8.33)	0.635[a]
Absent	22	18 (81.82)	4 (18.18)	
Multifocality				
Present	12	10 (83.33)	2 (16.67)	1.000[a]
Absent	22	19 (86.36)	3 (13.64)	
Extrathyroidal extension				
Present	15	13 (86.67)	2 (13.33)	1.000[a]
Absent	19	16 (84.21)	3 (15.79)	
Thyroid capsular invasion				
Present	16	13 (81.25)	3 (18.75)	1.000[a]
Absent	18	16 (88.89)	2 (11.11)	
BRAF mutation				
Positive	15	14 (93.33)	1 (6.67)	0.355[a]
Negative	19	15 (78.95)	4 (21.05)	

[a]P-value using Fisher's exact test. LNM, lymph node metastasis; FVPTC, follicular variant papillary thyroid cancer. BRAF, B-Raf proto-oncogene; cN1, all other patients (not cN0).

P=0.018) and temporary unilateral vocal cord palsy (3.5% vs. 1.2; P=0.039). Therefore, Conzo *et al* (15) stated that prophylactic central LND in patients with cN0 PTC should be used selectively.

To improve the pertinence of prophylactic central LND in patients with cN0 PTC and to avoid potential surgical morbidity, the data was further analyzed based on preoperative central lymph node status; the analyses were performed separately for patients with cN0 and cN1 PTC. Univariate analysis of all patients with PTC revealed that $BRAF^{V600E}$ was associated with central LNM in the 115 patients with cN1 PTC and the 172 patients with cN0 PTC. However, in the multivariate regression analysis, $BRAF^{V600E}$ was significant in neither patients with cN0 PTC nor in patients with cN1 PTC, suggesting that the effect of $BRAF^{V600E}$ as an indicator for performing prophylactic central LND was limited in this cohort of patients.

When the patients were subdivided into variant histological subtypes, in the univariate analysis $BRAF^{V600E}$ was a potent indicator for central LNM in patients with CVPTC, in addition to patients with cN0 CVPTC, but not for cN1 patients of this variant. In the multivariate regression, $BRAF^{V600E}$ was independently correlated with central LNM in all patients with CVPTC and patients with cN0 CVPTC, but not in cN1 patients of this subtype.

The aforementioned research demonstrating a correlation between $BRAF^{V600E}$ and central LNM (26-28) may not be representative of all patients with PTC. As demonstrated in the present study, $BRAF^{V600E}$ was independently associated with central LNM in overall patients with PTC, but when subdivided by histological variant, $BRAF^{V600E}$ was only significantly associated with the CVPTC subtype. In addition, when categorized by preoperative central lymph node status, $BRAF^{V600E}$ was only independently associated with central LNM in patients with cN0 CVPTC instead of in all patients with cN0 PTC. For patients with cN1 CVPTC, suspected LNM was already detected on preoperative ultrasonography and surgical removal of regional lymph nodes was typically recommended, thus the clinical significance of $BRAF^{V600E}$ or other clinicopathological features was limited.

Table IX. Univariate analyses of associations between of central LNM and clinicopathological features in patients with cN0 FVPTC.

Clinicopathological characteristic	Total, n	Central LNM (+), n (%)	Central LNM (-), n (%)	P-value
Total number	60	21	39	
Age at diagnosis, years				
≥45	29	11 (37.93)	18 (62.07)	
<45	31	10 (32.26)	21 (67.74)	0.645
Sex				
Female	50	19 (38.00)	31 (62.00)	0.468[a]
Male	10	2 (20.00)	8 (80.00)	
Pathological tumor size, cm				
>2	14	8 (57.14)	6 (42.86)	0.096[a]
≤2	46	13 (28.26)	33 (71.74)	
Underlying Hashimoto's thyroiditis				
Present	19	5 (26.32)	14 (73.68)	0.337
Absent	41	16 (39.02)	25 (60.98)	
Multifocality				
Present	16	7 (43.75)	9 (56.25)	0.392
Absent	44	14 (31.82)	30 (68.18)	
Extrathyroidal extension				
Present	17	8 (47.06)	9 (52.94)	0.218
Absent	43	13 (30.23)	30 (69.77)	
Thyroid capsular invasion				
Present	30	15 (50.00)	15 (50.00)	0.015
Absent	30	6 (20.00)	24 (80.00)	
BRAF mutation				
Positive	29	10 (34.48)	19 (65.52)	0.935
Negative	31	11 (35.48)	20 (64.52)	

[a]P-value using χ^2 test continuity correction. LNM, lymph node metastasis; FVPTC, follicular variant papillary thyroid cancer; BRAF, B-Raf proto-oncogene; cN0, subclinical nodal disease.

As a consequence, $BRAF^{V600E}$ was only associated with central LNM in a selected cohort of patients instead of all patients with PTC.

Other clinicopathological factors have been associated with central LNM. Tumor size has been demonstrated to be an important risk factor for central LNM in numerous studies (28,33). In the present study, tumor size was significantly different between central LNM-positive or -negative patients in overall patients with PTC and patients with CVPTC, in addition to in the univariate analysis. Furthermore, tumor size was an independent factor in the multivariate analysis. Upon further investigation using surgical approaches, tumor size was revealed to be associated with central LNM in overall patients with cN0 PTC and in patients with cN0 CVPTC, and was the only independent risk factor associated with central LNM in overall patients with cN0 PTC. Additionally, no significant differences were identified between patients with FVPTC and patients with AVPTC with or without LNM. Thyroid capsular invasion is another widely investigated risk factor for central LNM (26,27). The data from the present study demonstrated that thyroid capsular invasion was associated with central LNM in a number of comparison groups, as revealed by the univariate analysis results. Thyroid capsular invasion was the only significantly different factor for central LNM in patients with cN1 CVPTC.

Previous studies have demonstrated that underlying Hashimoto's thyroiditis has a protective effect for patients with PTC, and that it is associated with smaller tumor size, fewer LNM and improved prognosis (36,37). The data from the present study demonstrated that underlying Hashimoto's thyroiditis was negatively correlated with central LNM in overall patients with PTC and patients with CVPTC. When further analyzed, the difference was only significant in overall patients with cN0 PTC and in patients with cN0 CVPTC. Underlying Hashimoto's thyroiditis serves as an independent factor only in patients with cN0 CVPTC. Considering that a great fraction of patients with cN1 who received therapeutic LND had central LNM, the suspected regional lymph nodes should be routinely removed. Aggressive features, including larger tumor size, multifocality,

Table X. Univariate analyses of associations between central LNM and clinicopathological characteristics in patients with AVPTC.

Clinicopathological characteristic	Total, n	Central LNM (+), n (%)	Central LNM (-), n (%)	P-value
Total number	16	12	4	
Age at diagnosis, years				
≥45	5	4 (80.00)	1 (20.00)	
<45	11	8 (72.73)	3 (27.27)	0.755[a]
Sex				
Female	12	10 (83.33)	2 (16.67)	0.245[a]
Male	4	2 (50.00)	2 (50.00)	
Pathologic tumor size, cm				
>2	7	7 (100.00)	0 (0.00)	0.088[a]
≤2	9	5 (55.56)	4 (44.44)	
Underlying Hashimoto's thyroiditis				
Present	7	5 (71.43)	2 (28.57)	1.000[a]
Absent	9	7 (77.78)	2 (22.22)	
Multifocality				
Present	7	5 (71.43)	2 (28.57)	1.000[a]
Absent	9	7 (77.78)	2 (22.22)	
Extrathyroidal extension				
Present	5	4 (80.00)	1 (20.00)	1.000[a]
Absent	11	8 (72.73)	3 (27.27)	
Thyroid capsular invasion				
Present	5	4 (80.00)	1 (20.00)	1.000[a]
Absent	11	8 (72.73)	3 (27.27)	
BRAF mutation				
Positive	13	10 (76.92)	3 (23.08)	1.000[a]
Negative	3	2 (66.67)	1 (33.33)	

[a]P-value using Fisher's exact test. LNM, lymph node metastasis; AVPTC, aggressive variant papillary thyroid cancer; BRAF, B-Raf proto-oncogene.

extrathyroidal extension, thyroid capsular invasion and $BRAF^{V600E}$ became less important under such a high ratio of central LNM.

The present study has certain limitations. The sample size was not big enough, particularly for patients with s; only 16 patients with AVPTC in total were analyzed in the present study, which may affect the statistics. Additionally, on account of insufficient numbers of patients, the analyses of patients with cN0 and cN1 AVPTC were not performed separately. The association between $BRAF^{V600E}$ and other risk factors with long-term follow-up data in different subgroups require further investigation in order to unveil their prognostic function in different histological subtypes and preoperative central lymph node statuses.

In conclusion, the clinical significance of $BRAF^{V600E}$ for central LNM depends not only on PTC histological subtype, but also on preoperative central lymph node status, with the greatest significance in patients with cN0 CVPTC instead of all patients with PTC. Furthermore, $BRAF^{V600E}$ alone may not be able to accurately indicate the central lymph node status. Hence, other associated factors should also be recognized.

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