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The thyroid imaging reporting and data system on US, but not the BRAF^{V600E} mutation in fine-needle aspirates, is associated with lateral lymph node metastasis in PTC

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Abstract

The majority of patients with papillary thyroid carcinoma (PTC) have an excellent prognosis, but some show poorer outcomes and would benefit from adjunctive prognostic tools. The B-Raf proto-oncogene, serine/threonine kinase (BRAF)^{V600E} mutation, either based on both its presence or its quantitative measurement, and ultrasound (US) features may serve as a prognostic marker. The aim of this study was to investigate (1) the association between clinical-pathologic prognostic factors and the BRAF^{V600E} mutation found in fine-needle aspirates, based on both its presence and its corresponding cycle threshold (*Ct*) value, and (2) the association between prognostic factors and suspicious US features classified by the thyroid imaging reporting and data system (TIRADS) in PTC.

Two-hundred fifty-eight consecutive patients with PTC > 1 cm and who underwent preoperative US-guided fine-needle aspiration were included in this retrospective study. Clinical-pathologic variables were compared between patients with and without the BRAF^{V600E} mutation. Multivariate analyses were performed to investigate (1) the association between clinical-pathologic prognostic factors and the BRAF ^{V600E}mutation found in fine-needle aspirates, based on both its presence and corresponding *Ct* values, and (2) the association between prognostic factors and suspicious TIRADS US features.

BRAF^{V600E}-positive patients had a higher proportion of multiple tumors ($P=0.017$). The number of suspicious US features classified by the TIRADS was an independent factor for predicting lateral lymph node metastasis, both in all 258 patients (odds ratio [OR] = 1.902, $P=0.005$) and in 214 BRAF^{V600E}-positive patients (OR = 1.686, $P=0.037$). The BRAF^{V600E} mutation status or BRAF^{V600E}*Ct* values were not associated with any of the clinical-pathologic prognostic factors.

In conclusion, a higher number of suspicious US features classified by the TIRADS, but not the BRAF^{V600E} mutation, are associated with lateral lymph node metastasis in patients with PTC, and can aid in the preoperative identification of patients at increased risk of lateral lymph node metastasis.

Abbreviations: CIs = confidence intervals, *Ct* = cycle threshold, OR = odds ratio, PTC = papillary thyroid carcinoma, TIRADS = thyroid imaging reporting and data system, US = ultrasound.

Keywords: BRAF^{V600E} mutation, lateral lymph node metastasis, papillary thyroid carcinoma, thyroid imaging reporting and data system, ultrasound

1. Introduction

Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy and most patients have a favorable prognosis.[1,2] However, a small subset of PTCs show aggressive behavior and ~13.3% of patients experience recurrence, with some patients still dying from thyroid cancer.[1] Previous research has focused on identifying and establishing potential markers for risk stratification in PTC.[3,4] The B-Raf proto-oncogene, serine/threonine kinase (BRAF)^{V600E} mutation has been one of the most extensively investigated mutations, and its clinical importance lies in the fact that it is the most common genetic alteration in PTC and in its reported association with poor prognostic factors and patient outcomes.[5–7]

However, previous studies based on the presence or absence of the BRAF^{V600E} mutation have shown conflicting results regarding its role as a prognostic marker.[8–10] Recently, the controversial association between the presence of the BRAF^{V600E} mutation and prognostic factors has been partly attributed to the heterogeneous distribution of the BRAF^{V600E} mutation within

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tumors.[11] Recent studies utilizing pathologic tumor specimens have reported that a high allelic percentage of $BRAF^{V600E}$ mutation in PTC was associated with lymph node metastasis, extrathyroidal extension, and recurrence.[12–14] However, these results were based on $BRAF^{V600E}$ mutation in surgically resected tumor specimens and thus, may not be directly applicable to preoperative clinical settings. In a previous study, the cycle threshold (Ct) values for the $BRAF^{V600E}$ mutation found in fine-needle aspirates obtained by a highly sensitive real-time polymerase chain reaction (PCR) technique, which are quantitative measurements of the $BRAF^{V600E}$ mutation, were reported to be associated with central lymph node metastasis in patients with papillary thyroid microcarcinoma.[15] Yet, it is unclear whether this association will also apply to PTCs >1 cm, as the majority of the tumor volume would be less likely included when performing US-guided fine-needle aspiration.

The role of ultrasound (US) features as a potential prognostic tool in PTC has also been investigated, with the presence of malignant-appearing US features having been associated with lymph node metastasis, extrathyroidal extension, higher stage, and recurrence in PTCs >1 cm.[16,17] Recently, the thyroid imaging reporting and data system (TIRADS) by Kwak et al[18] which is based on the number of suspicious US features, has been shown to be accurate in malignancy risk stratification of thyroid nodules and simple to apply to clinical practice. However, its association with established prognostic factors in patients with PTC has not yet been examined.

Therefore, the aim of this study was to investigate (1) the association between clinical-pathologic prognostic factors and the $BRAF^{V600E}$ mutation found in fine-needle aspirates of PTCs, based on both its presence and its corresponding Ct value, and (2) the association between prognostic factors and suspicious US features classified by the TIRADS in a $BRAF^{V600E}$-prevalent population.

2. Material and methods

2.1. Study population

Our institutional review board approved this retrospective study and the requirement for informed consent was waived. Between December 2010 and June 2014, 1253 patients were diagnosed with thyroid cancer by preoperative US-guided fine-needle aspiration with simultaneous $BRAF^{V600E}$ mutation testing using real-time PCR or dual-priming oligonucleotide (DPO)-based multiplex PCR, and underwent surgery at our institution. Of the 1253 patients, 913 patients diagnosed with papillary thyroid microcarcinoma, 46 patients with PTC of other variants, and 36 patients who underwent $BRAF^{V600E}$ mutation testing using DPO-based multiplex PCR were excluded. Patients who were (1) diagnosed with conventional PTC > 1 cm and (2) underwent preoperative $BRAF^{V600E}$ mutation testing using real-time PCR with fine-needle aspirates were included. A total of 258 patients finally composed our study population, including 200 women (median age, 49 years; range, 16–83 years) and 58 men (median age, 41.5 years; range, 23–69 years).

2.2. US examination and TIRADS

Thyroid US and US-guided fine-needle aspiration were performed using a 5 to 12 MHz linear-array transducer (iU22; Philips Medical Systems, Bothell, Washington, DC) or a 6 to 14 MHz linear probe (EUB-7500, Hitachi Medical, Tokyo, Japan). US examinations were performed by 1 of 21 board-certified radiologists with 1 to 20 years of experience in thyroid imaging. The same radiologists performed US-guided fine-needle aspiration following the US examinations, and prospectively recorded the US features of thyroid nodules at the time of fine-needle aspiration. The nodule size was recorded based on the largest diameter on US. The internal components were classified as solid, predominantly solid (>50% solid for a mixed nodule), and predominantly cystic (>50% cystic for a mixed nodule). Echogenicity was classified as hyperechogenicity, isoechogenicity, hypoechogenicity, or marked hypoechogenicity.[19] Margins were classified as well-circumscribed, microlobulated, or irregular. Calcifications were categorized as microcalcifications (≤1 mm in diameter; tiny, punctuate, hyperechoic foci either with or without acoustic shadows), macrocalcifications, or no calcification. Shape was classified as taller than wide (greater in its anteroposterior dimension than in its transverse dimension) or wider than tall. US features which were considered suspicious for malignancy included marked hypoechogenicity, microlobulated or irregular margins, microcalcifications, or taller-than-wide shape. When thyroid nodules showed at least one of the suspicious US features, they were assessed as "suspicious." When thyroid nodules showed no suspicious US features, they were assessed as "probably benign." US-guided fine-needle aspiration was performed on either the nodule with suspicious US features or the largest probably benign nodule.

A TIRADS category was assigned to each nodule based on the number of the following US features—solidity, hypoechogenicity or marked hypoechogenicity, microlobulated or irregular margins, microcalcifications, and taller-than-wide shape.[18] Thyroid nodules with 1, 2, 3 or 4, or 5 of the above-mentioned US features were classified as categories 4a, 4b, 4c or 5, respectively. The number of the above-mentioned US features, which were classified as suspicious based on the TIRADS, was recorded and used for statistical analysis.

2.3. US-guided fine-needle aspiration and $BRAF^{V600E}$ mutation analysis

US-guided fine-needle aspiration was performed using a 23-gauge needle attached to a 2-mL disposable plastic syringe, with a freehand technique. Each lesion was aspirated at least twice. Materials obtained from aspiration were expelled onto a glass slide and smeared. After cytological preparation, the material remaining in the syringe was collected for $BRAF^{V600E}$ mutation testing, which was performed when requested for by the referring physician.

Real-time PCR was performed using the Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA) under the following cycle conditions; denaturation at 50°C for 2 minutes (1 cycle), 95°C for 10 minutes (1 cycle), and 95°C for 15 seconds (1 cycle), and one step of annealing and elongation at 62°C for 45 seconds (40 cycles). The Real-Q BRAFV600E Detection Kit (BioSewoom, Korea) was used to carry out the PCR reactions. This is a ready-to-use kit for the detection of the BRAF V600E somatic mutation in the BRAF oncogene in a background of wild-type genomic DNA using a multiplex real-time PCR assay based on the TaqMan MGB probe system. The BRAF mutation assay is labeled with VIC, and its amplification is detected by measuring the VIC fluorescence in the AB 7500 system. The internal control assay is labeled with FAM and is used to test for nucleic acid isolation and possible PCR inhibition, by amplifying a region of exon 8 of the BRAF gene. The 242 base pair of the partial BRAF gene containing the V600E region was amplified

from the human melanoma cell line, SK-MEL-28 (ATCC, Manassas, VA) with the BRAF mutation, and was inserted into the pZEM-T Easy Vector (Promega, Madison, WI) to produce the BRAF plasmid DNA. Analytical sensitivity was assessed using the BRAFV600E mutation plasmid clone and the 95% positive cut-off value (limit of detection) was calculated at 21.5 copy/μL by Probit analysis.[20]

The Ct was defined as the number of amplification cycles at which the level of fluorescent signal exceeds the threshold for the presence of the $BRAF^{V600E}$ mutation. The cut-off value for $BRAF^{V600E}$ mutation positivity was set to Ct 40, according to our previous report.[20] This cut-off value was determined by using the average Ct value obtained from 100 repeats of low-positive concentrations of the BRAF plasmid DNA, for which a positive rate of 100% was achieved. The Ct value is determined from a log-linear plot of the PCR signal versus the cycle number and is inversely related to the $BRAF^{V600E}$ mRNA level. Therefore, a low Ct value corresponds to a higher mRNA level.[21]

2.4. Surgical procedure

Total or near-total thyroidectomy was performed in patients with diagnosed or suspected multiple tumors, extrathyroidal invasion, or lymph node metastasis upon preoperative evaluation or intraoperative findings. Central compartment neck dissection was routinely performed in all of the patients, including the paratracheal, pretracheal, and prelaryngeal lymph nodes. Bilateral central compartment neck dissection was performed in patients undergoing total or near-total thyroidectomy and ipsilateral central compartment neck dissection was performed in patients undergoing hemithyroidectomy. Lateral compartment neck dissection was selectively performed in patients diagnosed with LNM on preoperative US-guided fine-needle aspiration. If suspicious lymph nodes were found during surgery, intraoperative frozen biopsy was performed. Lateral neck compartments including levels 2, 3, 4, and anterior 5 were dissected in patients confirmed to have lateral lymph node metastasis.

Of the 258 PTC patients, 244 (94.6%) patients underwent total or near-total thyroidectomy and 14 (5.4%) patients underwent hemithyroidectomy. Forty-three (16.7%) patients underwent therapeutic lateral compartment neck dissection. Information on tumor size, multiplicity including both uni- and bilateral tumor foci, extrathyroidal extension, and the presence of central and lateral lymph node metastasis were obtained from final surgical pathology reports.

2.5. Statistical analysis

The demographic, clinical-pathologic characteristics and the number of suspicious TIRADS US features were compared between patients with and without $BRAF^{V600E}$ mutation with the χ^2 test or Fisher's exact test for categorical variables and the Mann–Whitney *U* test for continuous variables. Multivariate logistic regression analysis was performed to identify independent factors for extrathyroidal extension, multiplicity, and central and lateral lymph node metastasis including the $BRAF^{V600E}$ mutation status, i.e., the presence or absence of the $BRAF^{V600E}$ mutation, as a covariate.

For the $BRAF^{V600E}$-positive patients, $BRAF^{V600E}$Ct values were compared according to clinical-pathologic prognostic factors using the Mann–Whitney *U* test. The Spearman's correlation coefficient (r) was used to evaluate the association between the BRAF Ct values and tumor size. We performed a separate multivariate logistic regression analysis in the $BRAF^{V600E}$-positive patients to identify independent factors for exthrathyroidal extension, multiplicity, and central and lateral lymph node metastasis, including the $BRAF^{V600E}$Ct value as a covariate. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

A two-tailed *P* value of <0.05 was defined as a statistically significant difference. Statistical analyses were performed with SPSS statistical software (SPSS for Windows, version 20.0; IBM Corporation, Armonk, NY).

3. Results

The median tumor size was 13 mm (range, 11–40 mm) for the 258 PTCs. Of the 258 patients, 214 patients (82.9%) had the $BRAF^{V600E}$ mutation which was confirmed through real-time PCR testing from fine-needle aspirates. Among the study population, 123 (47.7%) patients had pathologically confirmed central lymph node metastasis, 43 (16.7%) had lateral lymph node metastasis, 163 (63.2%) had extrathyroidal extension, and 87 (33.7%) patients had multiple tumors.

3.1. $BRAF^{V600E}$ mutation status, TIRADS and clinical-pathologic prognostic factors in PTCs

$BRAF^{V600E}$-positive patients had a significantly higher proportion of multiple tumors at surgical resection ($P=0.017$), and had a higher number of suspicious TIRADS US features with borderline significance ($P=0.061$) (Table 1). At multivariate analysis, the number of suspicious US features classified by the

Table 1

Comparison of clinical-pathologic characteristics and number of suspicious TIRADS US features according to the $BRAF^{V600E}$ mutation status in 258 papillary thyroid carcinoma patients.

	$BRAF^{V600E}$ + (n = 214)	$BRAF^{V600E}$– (n = 44)	*P* value
Age, y, median (range)	48 (17–83)	46 (16–69)	0.584
Sex			0.052
Female	161 (75.2%)	39 (88.6%)	
Male	53 (24.8%)	5 (11.4%)	
Tumor size, mm, median (range)	13 (11–40)	12.5 (11–40)	0.975
Central LNM			0.746
Positive	103 (48.1%)	20 (45.5%)	
Negative	111 (51.9%)	24 (54.5%)	
Lateral LNM			0.767
Positive	35 (16.4%)	8 (18.2%)	
Negative	179 (83.6%)	36 (81.8%)	
Extrathyroidal extension			0.945
Positive	135 (63.1%)	28 (63.6%)	
Negative	79 (36.9%)	16 (36.4%)	
Multiplicity			0.017
Positive	79 (36.9%)	8 (18.2%)	
Negative	135 (63.1%)	36 (81.8%)	
No. of suspicious TIRADS US features, median (range)	4 (1–5)	4 (0–5)	0.061
TIRADS category			0.148
3	0 (0%)	1 (2.3%)	
4a	2 (0.9%)	1 (2.3%)	
4b	13 (6.1%)	4 (9.1%)	
4c	144 (67.3%)	30 (68.2%)	
5	55 (25.7%)	8 (18.2%)	

$BRAF^{V600E}$ + = $BRAF^{V600E}$ mutation-positive, $BRAF^{V600E}$– = $BRAF^{V600E}$ mutation-negative, LNM = lymph node metastasis, No. = number, TIRADS = thyroid imaging reporting and data system, US = ultrasound.

Table 2

Multivariate logistic regression analysis for clinical-pathologic prognostic factors based on the presence or absence of the BRAF mutation in 258 papillary thyroid carcinoma patients.

	Dependent variable					
	Extrathyroidal extension		Central LNM		Lateral LNM	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.007 (0.987, 1.028)	0.489	0.976 (0.956, 0.996)	0.017	0.986 (0.959, 1.013)	0.297
Male sex	0.409 (0.217, 0.770)	0.006	1.819 (0.947, 3.491)	0.072	1.701 (0.744, 3.890)	0.208
Tumor size, mm	1.012 (0.964, 1.062)	0.637	1.032 (0.982, 1.084)	0.212	1.095 (1.034, 1.159)	0.002
BRAF positivity	1.038 (0.508, 2.124)	0.918	0.994 (0.484, 2.042)	0.987	0.699 (0.266, 1.835)	0.467
Central LNM	0.943 (0.545, 1.632)	0.835			2.265 (1.055, 4.863)	0.036
Lateral LNM	1.527 (0.706, 3.302)	0.282	2.235 (1.051, 4.753)	0.037		
Extrathyroidal extension			0.948 (0.550, 1.636)	0.849	1.534 (0.707, 3.328)	0.278
Multiplicity	1.014 (0.577, 1.781)	0.962	1.550 (0.893, 2.692)	0.119	1.663 (0.795, 3.478)	0.176
No. of suspicious TIRADS US features	1.219 (0.911, 1.631)	0.182	1.105 (0.826, 1.476)	0.502	1.902 (1.219, 2.968)	0.005

BRAF = B-Raf proto-oncogene, serine/threonine kinase, CI = confidence interval, LNM = lymph node metastasis, No. = number, OR = odd ratio, TIRADS = thyroid imaging reporting and data system, US = ultrasound.

TIRADS was an independent factor for predicting lateral lymph node metastasis (OR = 1.902, 95% CI: 1.219–2.968 [*P* = 0.005]) (Table 2). The $BRAF^{V600E}$ mutation status was not significantly associated with any of the clinical-pathologic prognostic factors. Lateral lymph node metastasis was also significantly associated with larger tumor size (OR = 1.095, 95% CI: 1.034–1.159 [*P* = 0.002]) and central lymph node metastasis (OR = 2.265, 95% CI: 1.055–4.863 [*P* = 0.036]). Extrathyroidal extension was significantly associated with female sex (OR = 2.447, 95% CI: 1.299–4.609 [*P* = 0.006]). Central lymph node metastasis was associated with younger age (OR = 0.976, 95% CI: 0.956–0.996 [*P* = 0.017]) and lateral lymph node metastasis (OR = 2.235, 95% CI: 1.051–4.753 [*P* = 0.037]).

3.2. $BRAF^{V600E}$ Ct value, TIRADS, and clinical-pathologic prognostic factors in BRAF-positive PTCs

For the 214 $BRAF^{V600E}$-positive PTC patients, $BRAF^{V600E}$ Ct values and the number of suspicious TIRADS US features were compared according to clinical-pathologic prognostic factors. There was no significant difference of the quantitative expression of the $BRAF^{V600E}$ mutation, expressed as a *Ct* value (Table 3). Patients with lateral lymph node metastasis had a higher number of suspicious TIRADS US features with borderline significance (*P* = 0.064). $BRAF^{V600E}$ Ct values showed a weak but significant negative correlation with tumor size ($r = -0.207$, $P = 0.002$) (Fig. 1).

At multivariate analysis, the number of suspicious US features classified by the TIRADS was an independent factor for predicting lateral lymph node metastasis among $BRAF^{V600E}$-positive PTC (OR = 1.686, 95% CI: 1.031–2.758 [*P* = 0.037]) (Table 4). BRAF Ct values were not significantly associated with any of the clinical-pathologic prognostic factors. Lateral lymph node metastasis also showed association with a larger tumor size (OR = 1.112, 95% CI: 1.038–1.192 [*P* = 0.003]) and central lymph node metastasis (OR = 2.612, 95% CI: 1.108–6.157 [*P* = 0.028]). Extrathyroidal extension was significantly associated with female sex (OR = 2.592, 95% CI: 1.317–5.098 [*P* = 0.006]). Central lymph node metastasis was significantly associated with younger age (OR = 0.973, 95% CI: 0.951–0.997 [*P* = 0.025]), male sex (OR = 2.126, 95% CI: 1.059–4.270 [*P* = 0.034]), and lateral lymph node metastasis (OR = 2.620, 95% CI: 1.122–6.118 [*P* = 0.026]).

Table 3

Comparison of quantitative expression of the BRAF mutation and number of suspicious TIRADS US features according to clinical-pathologic prognostic factors in 214 $BRAF^{V600E}$-positive papillary thyroid carcinoma patients.

	$BRAF^{V600E}$ *Ct* value*	*P* value	No. of suspicious TIRADS US features*	*P* value
Sex		0.702		0.234
Female	27.6 (24.1–38.3)		4 (1–5)	
Male	27.3 (24.6–38.2)		4 (1–5)	
Central LNM		0.542		0.384
Positive	27.3 (24.1–38.3)		4 (1–5)	
Negative	27.7 (24.3–38.3)		4 (2–5)	
Lateral LNM		0.832		0.064
Positive	27.3 (24.9–38.3)		4 (2–5)	
Negative	27.5 (24.1–38.3)		4 (1–5)	
Extrathyroidal extension		0.311		0.358
Positive	28.3 (24.3–38.3)		4 (1–5)	
Negative	27.3 (24.1–38.2)		4 (1–5)	
Multiplicity		0.995		0.748
Positive	27.6 (24.7–38.3)		4 (1–5)	
Negative	27.5 (24.1–38.3)		4 (1–5)	

BRAF = B-Raf proto-oncogene, serine/threonine kinase, LNM = lymph node metastasis, No. = number, TIRADS = thyroid imaging reporting and data system, US = ultrasound.
* Data are expressed as median and range within parentheses.

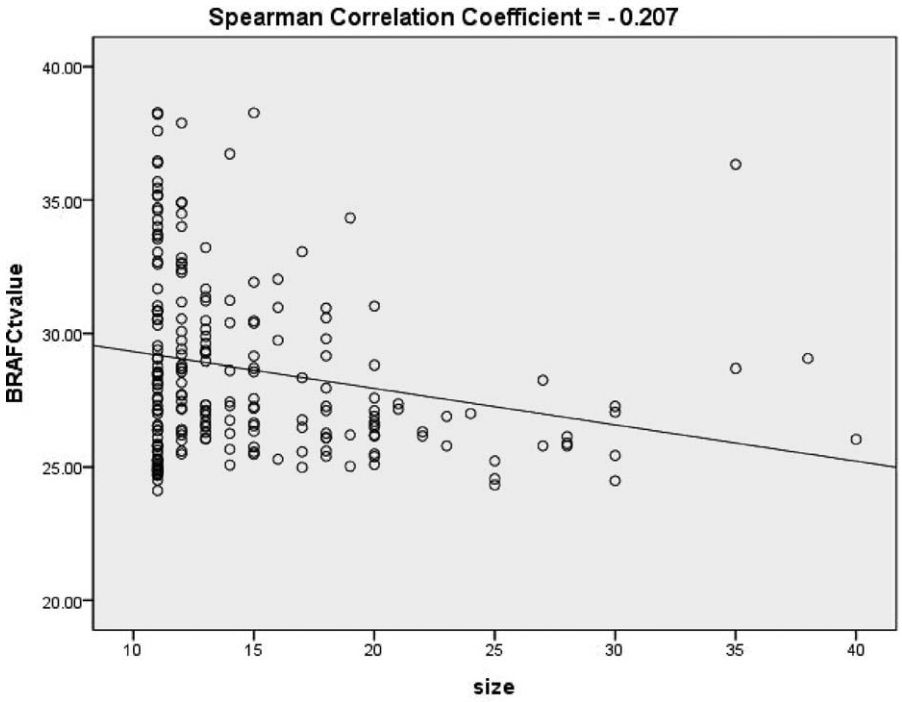

Figure 1. Scatter plot for the correlation between $BRAF^{V600E}$Ct values and tumor size ($r = -0.207$, $P = 0.002$) in the 214 $BRAF^{V600E}$-positive patients. $BRAF^{V600E}$ = B-Raf proto-oncogene, serine/threonine $kinase^{V600E}$, *C*t = cycle threshold.

4. Discussion

We found that the number of suspicious US features classified by the TIRADS was independently associated with lateral lymph node metastasis in PTC >1 cm, both in the entire study population and in the $BRAF^{V600E}$-positive subpopulation. In addition, we found that the quantitative expression of the $BRAF^{V600E}$ mutation found in preoperatively obtained fine-needle aspirates, expressed as a *Ct* value, was not associated with prognostic factors in PTC in a $BRAF^{V600E}$-prevalent population.

Although the majority of PTCs follow an indolent course, ~2.2% to 20.6% of patients experience recurrence after surgery.[2,22,23] In a recent large cohort study conducted in Denmark, 11.4% (108 of 944) of PTCs >1 cm had recurrence and 7.6% (68 of 944) died from thyroid cancer during a median follow-up period of 7.9 years.[2] Among clinical-pathologic variables, nodal metastasis has been reported as the strongest predictor of recurrence in patients with PTC, and patients with cervical lymph node metastasis have shown higher recurrence rates and disease-specific mortality.[2,23,24] Therefore, tools that help identify patients at increased risk of cervical lymph node metastasis may also contribute to improved outcomes through facilitation of earlier detection and treatment. Previous researchers reported that the disease-free survival rates at 5 years after initial surgery for PTCs >1 cm have significantly increased from 73% to 91%, and this change was largely attributed to the earlier diagnosis and treatment of lymph node metastasis.[25] Thus, our study results suggest that the number of suspicious TIRADS US features may be used in the preoperative risk stratification of PTC, by assisting identification of patients at increased risk for

Table 4

Multivariate logistic regression analysis for clinical-pathologic prognostic factors based on the quantitative expression of the $BRAF^{V600E}$ mutation in 214 $BRAF^{V600E}$-positive papillary thyroid carcinoma patients.

	Dependent variable					
	Extrathyroidal extension		Central LNM		Lateral LNM	
Variables	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age	1.011 (0.987–1.035)	0.384	0.973 (0.951–0.997)	0.025	0.991 (0.959–1.023)	0.564
Male sex	0.386 (0.196–0.759)	0.006	2.126 (1.059–4.270)	0.034	1.050 (0.417–2.644)	0.918
Tumor size, mm	1.040 (0.979–1.104)	0.204	0.974 1.033 (0.974–1.095)	0.282	1.112 (1.038–1.192)	0.003
BRAF *Ct* value	1.063 (0.971–1.165)	0.187	0.966 (0.883–1.056)	0.443	1.046 (0.923–1.185)	0.483
Central LNM	1.039 (0.567–1.903)	0.902			2.612 (1.108–6.157)	0.028
Lateral LNM	1.269 (0.544–2.958)	0.582	2.620 (1.122–6.118)	0.026		
Extrathyroidal extension			1.045 (0.570–1.914)	0.888	1.280 (0.547–2.994)	0.569
Multiplicity	1.034 (0.568–1.884)	0.912	1.340 (0.742–2.422)	0.332	1.612 (0.732–3.547)	0.235
No. of suspicious TIRADS US features	1.112 (0.798–1.552)	0.530	1.081 (0.774–1.508)	0.648	1.686 (1.031–2.758)	0.037

BRAF = B-Raf proto-oncogene, serine/threonine kinase, CI = confidence interval, LNM = lymph node metastasis, OR = odd ratio, TIRADS = thyroid imaging reporting and data system, US = ultrasound.

lateral cervical lymph node metastasis and therefore contributing to more precise diagnosis and individualized surveillance.

Our findings are consistent with several previous studies on the association between US features and prognostic factors in PTC. Previous investigators have found that malignant-appearing PTCs, i.e., PTCs that had at least one suspicious US feature, more frequently had aggressive prognostic factors than benign-appearing PTCs and that the presence of suspicious US features was independently associated with recurrence.[16,17] In one study, a greater number of suspicious US findings was associated with multifocality, extrathyroidal extension, lymph node metastasis, and a high stage.[16] Although the specific US features that are classified as suspicious by the TIRADS differ from the two prior studies, our results also support the potential role of US features as a prognostic tool in PTC. The number of suspicious TIRADS US features was independently associated with only lateral lymph node metastasis, not central lymph node metastasis, and therefore may be a more useful tool in the preoperative setting. As the number of suspicious TIRADS US features increases, more meticulous lateral neck evaluation should be performed at preoperative neck US, which would allow better surgical planning through the additional detection of lateral cervical lymph node metastasis.

The role of the $BRAF^{V600E}$ mutation as a prognostic marker of PTC is still controversial. The presence of the $BRAF^{V600E}$ mutation has been associated with poor clinical-pathological characteristics and poor outcome,[6,26–28] whereas others have failed to demonstrate a significant association.[8,9,29] In our study, the presence of the $BRAF^{V600E}$ mutation, i.e., its qualitative detection, showed no significant association with variable clinical-pathological factors in PTC, which is consistent with several studies.[8,9,29] Such conflicting results have recently been partly attributed to the heterogeneous distribution of the $BRAF^{V600E}$ mutation in tumors, as PTCs generally consist of a mixture of tumor cells with wild type and mutant $BRAF^{V600E}$.[11] Previous reports based on surgically resected PTC specimens have shown that a high mutant allelic frequency was associated with disease recurrence or extrathyroidal invasion,[12,30] but only one study reported a significant association with lymph node metastasis.[13] Thus, studies on the association between quantitative measurements of the $BRAF^{V600E}$ mutation from surgical specimens have also shown variable results. In a recent report, we have demonstrated that real-time PCR *Ct* values for the $BRAF^{V600E}$ mutation in fine-needle aspirates were associated with central lymph node metastasis in papillary thyroid microcarcinoma.[15] However, our results suggest that this association does not apply to PTCs >1 cm, and that $BRAF^{V600E}$*Ct* values cannot be utilized as a preoperative predictor for lymph node metastasis in this subgroup. A likely explanation is that only a portion of the tumor would have been sampled during US-guided fine-needle aspiration in larger PTCs, and therefore, the *Ct* values are less likely to sufficiently represent the entire tumor. Nonetheless, there seems to be a correlation between tumor volume and the quantitative expression of the $BRAF^{V600E}$ mutation, as our study showed a weak but significant correlation with $BRAF^{V600E}$*Ct* values and tumor size, which is similar to previous studies assessing the mutant $BRAF^{V600E}$ allelic percentage or its relative expression in PTC.[12,13,21,30]

Our study had some limitations. First, this was a retrospective study from a single institution and we included patients who underwent US-fine-needle aspiration and simultaneous $BRAF^{V600E}$ testing. Thus, a selection bias in our study population is inevitable. Second, although the *Ct* value is a quantitative expression of gene mutation in real-time PCR, comparisons relative to a reference gene are required for accurate relative quantification.[31] However, the routine obtainment of such data is impractical in clinical practice and other studies have directly applied the *Ct* value as a relative measure of mRNA concentration levels.[32] Finally, we did not investigate the correlation between quantitative measurements of the $BRAF^{V600E}$ mutation in fine-needle aspirates and those from surgical specimens. Although the $BRAF^{V600E}$ mutation status shows high concordance between cytological and histological samples, to our knowledge, comparison based on its quantitative measurement has not yet been reported.[33] Further research on the correlation between quantitative measurements of the BRAFV600E mutation between cytological and histological samples will provide more information on whether quantitative measurements of the BRAFV600E mutation in fine-needle aspirates, or only those from surgical specimens, can serve as a potential prognostic marker in PTC.

In conclusion, the number of suspicious US features classified by the TIRADS, but not the $BRAF^{V600E}$ mutation in fine-needle aspirates, was associated with lateral lymph node metastasis in patients with PTC. The TIRADS may have important implications for preoperatively identifying patients at increased risk of lateral lymph node metastasis.

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