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Can Suspicious Ultrasound Features Predict BRAFV600E Status in Papillary Thyroid Cancer?

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BRAFV600E · Thyroid cancer · Ultrasound · Screening

Abstract
Background: Papillary thyroid carcinoma (PTC) can be predicted from certain suspicious ultrasound (US) features of thyroid nodules. The aim of this study was to examine if these suspicious features can predict the more aggressive PTC associated with B-type Raf kinase (BRAFV600E) mutation. ***Methods:*** This was a retrospective review of prospectively collected data on patients with PTC and known BRAFV600E status. All patients underwent preoperative US by the same surgeon who performed all the operations. We divided patients into BRAFV600E positive and negative groups. All ultrasonographic data were collected including nodule size, echogenicity, solid or cystic nature, presence of calcifications, irregular margins, and internal vascularity. ***Results:*** Of 141 patients with PTC, BRAFV600E mutation was detected in 48 (34.0%) patients. There was no significant difference in nodule size (2.06 cm ± 1.37 vs. 2.15 cm ±1.55, p = 0.75) between BRAFV600E positive and negative groups. BRAFV600E positivity was associated with higher rates of hypoechogenicity (57.5% vs. 36.6, p = 0.02), calcifications (48.9 vs. 19.4%, $p < 0.01$), and irregular margins (21.3 vs. 6.5%, $p < 0.01$). There was no significant difference in the noncystic nature or internal vascularity between BRAFV600E positive and negative groups. The presence of all suspicious US features is associated with a positive predictive value of 100.0%. In the absence of all suspicious features, the negative predictive value was 84.2%. When suspicious lymph nodes (LNs) detected by preoperative US were compared, there was no significant difference between BRAFV600E positive and negative groups (30.6 vs. 21.7%, p = 0.35). ***Conclusion:*** The presence of multiple suspicious US findings of thyroid nodules can predict the BRAFV600E mutation status of papillary thyroid cancer nodules. The highest accuracy overall (93.2%) was achieved by combining calcification, irregular margins, and hypoechogenicity with extrathyroidal extension and LN metastasis. Future multi-institutional studies are warranted to help surgeons with risk stratification and operative planning for patients with papillary thyroid cancer.

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Introduction

The incidence of thyroid cancer has been steadily increasing in the United States. This has been attributed in part due to the increased detection of small papillary thyroid cancer (PTC) [1]. As most thyroid cancers initially present as thyroid nodules, the American Thyroid Association and the American Association of Clinical Endocrinologists have set recommendations for the systematic evaluation of thyroid nodules [2, 3]. Their guidelines for the management of thyroid nodules include an initial comprehensive history and physical and a thorough initial clinical evaluation, which includes a cervical ultrasound (US).

As the management of malignant thyroid nodules is mostly surgical, it should be differentiated from benign nodules, which can be managed conservatively. Suspicious US features described to select nodules with malignancy potential to undergo fine needle aspiration (FNA) included hypoechogenicity, microcalcifications, irregular margins, non-cystic composition, loss of the halo, taller-than-wide shape, and internal hypervascularity [4–6]. Preoperative US can also provide information about contralateral lobe lesions or suspicious lymph nodes (LNs) that can aid in making decisions about the extent of thyroidectomy and in whether lymphadenectomy should be considered.

B-type Raf kinase (BRAFV600E) mutation on exon 15 results from mutation in BRAF genes leading to the substitution of glutamic acid with valine at amino acid 600 (BRAFV600E mutation) on exon 15 [7]. It is an oncogene that activates the mitogen-activated protein kinase signaling pathway and tumorigenesis, and is considered the most common mutation in PTC, occurring in 29–83% of PTC cases [8]. BRAFV600E oncogene has been associated with more aggressive phenotypes of PTC with more prevalent extrathyroidal extension (ETE) and LN metastasis [9, 10]. Tumors with BRAFV600E mutation also show a higher risk of disease persistence and recurrence [9, 11, 12]. As a prognostic factor, BRAFV600E mutation can be useful in tailoring the initial extent of thyroid surgery and LN dissection in addition to the timing and frequency of follow-up [9, 11, 12]. Targeted therapies for BRAFV600E positive PTC may also be used as an adjuvant therapy for metastatic PTC [10].

Few previous studies have correlated preoperative suspicious US features to the BRAF mutation, which as a prognostic factor can aid in risk stratification and surgical management planning. Kabaker et al. [13] found a significant association between BRAFV600E mutation and suspicious US features of thyroid nodules including hypoechogenicity, ill-defined margins, micro/macrocalcifications, absent halo, and taller-than-wide shape. Two other previous studies found a correlation between BRAFV600E mutation and small tumor size and high TNM stages (III and IV) [14] and fewer calcifications compared to BRAFV600E negative tumors [15]. These studies did not demonstrate a significant association between BRAFV600E mutation and the other suspicious US features.

In this study, we examined the possible correlation of suspicious US features of thyroid nodules diagnosed as PTC with the presence of the more aggressive phenotype of PTC associated with BRAFV600E mutation.

Methods

Under the Institutional Review Board approval, we conducted this retrospective study at the Tulane University Medical Center. We reviewed the charts of consecutive patients who underwent thyroidectomy for thyroid cancer from January 2008 to July 2016. We included patients who met our inclusion criteria of preoperative US in the 1 month before surgery, PTC on postsurgical histopathology, and molecular testing of surgical pathology specimens for BRAFV600E mutation. All surgeries were performed at our institution by a single endocrine surgeon (E.K.). We used a US scanner with 15 MHz transducer to perform diagnostic US examination. All US examinations were done by the same endocrine surgeon and a trained US technician using static and real-time imaging to document sonographic features of thyroid nodules and LNs. The nodules were imaged in sagittal and transverse planes and the vascularity was assessed by color Doppler US. Select demographics of the patients were collected. We reviewed the clinical, radiographic, and pathological parameters of our patients.

All surgical pathology reports were compared to the preoperative US studies to ensure that the PTC was in the same initially evaluated nodule. Any PTC that was found incidentally by histopathology and was not evaluated by US preoperatively was excluded. The primary tumors were evaluated for the BRAF mutation status. Central with or without lateral compartment LN dissections were performed if suspicious lymphadenopathy was found on preoperative US, metastatic PTC was confirmed on FNA.

We collected US characteristics of thyroid nodules including their size, echogenicity (hypoechoic, isoechoic, or hyperechoic) compared to surrounding normal thyroid parenchyma and anterior strap muscle, micro/macrocalcifications, margins (ill-defined or well-defined), composition (noncystic or have cystic component), and presence or absence of internal hypervascularity. We classified nodular US features as suspicious or not according to the previously described suspicious features: hypoechogenicity, the presence of micro/macrocalcifications, ill-defined margins, noncystic composition, and internal hypervascularity [4–6, 16]. Eggshell peripheral calcification was not considered a suspicious feature of thyroid nodules.

Table 1. Descriptive statistics and bivariable analysis based on BRAF

Covariate	Sample (n = 141)	BRAF (–) (n = 93)	BRAF (+) (n = 48)	p value
Age, years, mean ± SD	50.57 (14.52)	49.75 (14.63)	52.17 (14.30)	0.351
Gender, female, n (%)	105 (74.47)	68 (73.12)	37 (77.08)	0.609
Operation, n (%)				
Hemithyroidectomy	64 (45.39)	50 (53.76)	14 (29.17)	**0.005**
Completion thyroidectomy	23 (16.31)	16 (17.20)	7 (14.58)	0.690
Total thyroidectomy	15 (10.64)	8 (8.60)	7 (14.58)	0.275
Thyroidectomy + central neck dissection	24 (17.02)	13 (13.98)	11 (22.92)	0.181
Thyroidectomy + lateral neck dissection	3 (2.13)	0 (0.00)	3 (6.25)	**0.038**
Thyroidectomy + central and lateral	12 (8.51)	6 (6.45)	6 (12.50)	0.223
Nodule size, mean ± SD	2.12 (1.48)	2.15 (1.55)	2.06 (1.37)	0.753
ETE present, n (%)	15 (10.64)	6 (6.45)	9 (18.75)	**0.025**
LNM present, n (%)	35 (24.82)	18 (19.35)	17 (35.42)	**0.036**
Recurrence, n (%)	12 (8.51)	6 (6.45)	6 (12.50)	0.223
Hypo-echogenicity, n (%)	61 (43.57)	34 (36.56)	27 (57.45)	**0.019**
Calcification, n (%)	41 (29.29)	18 (19.35)	23 (48.94)	**<0.001**
Irregular margins, n (%)	16 (11.43)	6 (6.45)	10 (21.28)	**0.009**
All solid, n (%)	70 (50.00)	48 (51.61)	22 (46.81)	0.591
High vascularity, n (%)	55 (39.29)	32 (34.41)	23 (48.94)	0.096
LN USG suspicious, n (%)	29 (23.58)	18 (21.43)	11 (28.21)	0.410
Malignant-LN FNA, n (%)	16 (42.11)	8 (38.10)	8 (47.06)	0.578
Malignant-LN surgical, n (%)	43 (57.33)	23 (53.49)	20 (62.50)	0.435

We reviewed US characteristics for any LNs detected on cervical US to differentiate them as suspicious or not, and correlated them to the BRAF status of each patient to evaluate whether suspicious LN features on US are associated with BRAF-positive PTC. LNs were considered suspicious if they were round without fatty hilum, with calcification, or with cystic changes. The histopathology reports were reviewed to document any ETE or LN metastasis on final pathology.

Statistical methods included Student's t test for the continuous variables and Fisher's exact test for the categorical variables. Validity analysis was applied in the calculation of the sensitivity, specificity, positive predicative value, negative predicative value (NPV), accuracy, and positive likelihood ratio. The pre-test probability of positive BRAF was calculated based on the sample prevalence. Statistical significance was considered to be $\alpha = 0.05$. All analyses were carried out using SAS 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

There were 141 subjects meeting criteria to be included in this study, with an average age of 50.6 ± 14.5 years, with the majority of our study population being female (74.5%; Table 1). Sixty-four (45.4%) patients underwent thyroid lobectomy, 23 (16.3%) underwent completion thyroidectomy, 15 (10.6%) total thyroidectomy, 24 (17.0%) total thyroidectomy with central LN dissection, and 12 (8.5%) total thyroidectomy with concomitant central and lateral LN dissection. The average nodule size was 2.12 ± 1.48 cm. Fifteen (10.6%) patients had ETE on final histopathology and 35 (24.8%) had LN metastasis. Twelve of our patients underwent reoperations for recurrent LN disease; none showed subsequent recurrence. There was no distant metastasis in any of our patients. There was no cancer-related mortality encountered in our study sample.

Forty-eight (34.0%) of our patients were BRAFV600E positive, while the other 93 (66.0%) were BRAFV600E negative. There was no significant difference in the mean age between BRAFV600E positive (52.17 ± 14.30 years) and BRAFV600E negative patients (49.75 ± 14.63, p = 0.35; Table 1). The gender distribution was also similar: 77.1% females in BRAFV600E positive versus 73.1% in BRAFV600E negative group (p = 0.61). There was no significant difference in nodule size (2.06 cm ± 1.37 vs. 2.15 cm ± 1.55, p = 0.75) between BRAFV600E positive and negative groups. LN metastasis was significantly higher in the BRAF positive group (35.4 vs. 19.3%, p = 0.04), and in ETE between groups (p = 0.03). No cancer-specific mortality was detected in either group.

Compared to BRAF negative PTC, BRAF positive lesions were associated with higher rates of hypoechogenicity (57.4 vs. 36.6, p = 0.02), micro/macrocalcifications (48.9 vs. 19.3%, p < 0.01), and irregular margins

Table 2. Validity analysis for detecting positive BRAF by ultrasound features

Feature	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
1. Calcification	48.9	80.6	56.1	75.8	70.0
2. Irregular margins	21.3	93.5	62.5	70.2	69.3
3. Hypoechoic	57.4	63.4	44.3	74.7	61.4
4. Highly vascular	48.9	65.6	41.8	71.8	60.0
5. Non-cystic	46.8	48.4	31.4	64.3	47.9
1 + 2	20.8	96.0	62.5	79.1	77.8
1 + 2 + 3	20.0	97.9	66.7	85.5	84.5
1 + 2 + 3 + 4	14.3	100.0	100.0	84.2	84.6
1 + 2 + 3 + 4 + 5	33.3	100.0	100.0	80.0	81.8

PPV, positive predictive value; NPV, negative predictive value.

(21.3 vs. 6.4%, $p < 0.01$). No significant difference was found in noncystic nature of nodules (46.8 vs. 51.6%, $p = 0.59$) or internal hypervascularity (48.9 vs. 34.4%, $p = 0.10$) between groups. When suspicious LNs detected by preoperative US were compared, there was no significant difference between BRAF positive and negative groups (28.2 vs. 21.4%, $p = 0.41$; Table 1).

On validity analysis (Table 2), the combination of US features with the highest accuracy (84.6%) for correctly classifying BRAF positivity was micro/macrocalcifications, irregular margins, hypoechogenicity, and high vascularity (sensitivity 14.3%, specificity 100.0%). The likelihood for a PTC to be BRAF positive increased with the number of suspicious features on preoperative US up to this combination. The absence of all of the 5 US features had an accuracy of 62.1% (sensitivity 4.3%, specificity 91.4%). The highest accuracy overall (93.2%) was achieved by combining calcification, irregular margins, and hypoechogenicity with ETE and LN metastasis (sensitivity 33.3%, specificity 97.6%). Of all the US features predicting BRAF positivity in multivariable regression adjusting for age, sex, nodule size, ETE, LN metastasis, recurrence, and suspicious LN on US, only micro/macrocalcifications (OR 7.17, 95% CI 2.55–20.18; $p < 0.001$) and irregular margins (OR 4.87, 95% CI 1.20–19.74; $p = 0.027$) were significant predictors.

Discussion

To our knowledge, this is the first study to examine if suspicious US features of thyroid nodules can predict BRAF status for ETE and LN metastasis in PTC patients. Cervical US has been the initial workout for evaluating thyroid nodules because of its noninvasiveness and its ability to detect any additional nodules as well as concomitant thyroid, parathyroid, or LN pathology. In the case of thyroid cancer, US can be helpful in surgical decision making regarding the extent of thyroidectomy and whether cervical LN dissection is necessary. Nodules with suspicious US features are further evaluated with FNA, as they are more likely to be malignant [4–6]. Routine preoperative US helps in detecting nonpalpable LNs suspicious for metastasis that can be missed in physical exam, thus avoiding persistent disease and early reoperations [17, 18]. However, LN metastasis can still be found intraoperatively despite a negative physical exam and cervical US.

BRAFV600E is a mutation that has been associated with more aggressive PTC phenotypes with higher risk of ETE, LN metastasis, and higher TNM staging [9, 11, 13, 19]. However, other studies did not find a significant difference in ETE or LN metastasis between BRAF positive and BRAF negative PTC [15]. BRAFV600E mutation is considered an independent poor prognostic factor irrelevant to other factors of poor prognosis [20]. We found that BRAF positive PTC was associated with higher rate of LN metastasis and ETE. Recurrence rates and cancer-specific mortality were also reported to be higher in patients with BRAF positive PTC [10, 20]. We did not encounter any cancer-specific mortality in either BRAF positive or negative groups.

In our study, we found that BRAF positivity was associated with suspicious US features including hypoechogenicity, irregular margins, and micro/macrocalcifications. Internal hypervascularity did not show a significant association with BRAFV600E mutation. Kabaker et al. [13] similarly found that BRAF positive PTC was associated with hypoechogenicity, micro/macrocalcifications,

and irregular margins; in addition, it was associated with absent halo and taller-than-wide nodules but not with non-cystic composition. They found that 3 or more suspicious US features had a positive predicative value of 82%, while the absence of any suspicious features with negative BRAFV600E mutation had NPV of 88% for ETE and LN metastasis. In our experience, the absence of any US features had an NPV of 83.3% for BRAFV600E mutation, while the presence of at least 3 suspicious features had an accuracy of 84.5% in predicting BRAF positivity in PTC.

Another study found that there was no significant difference between BRAF positive and negative PTC in any sonographic feature except for calcifications, which were unexpectedly lower in BRAF positive tumors [15]. A study done on micro PTC found no significant association between suspicious US features and BRAFV600E mutation [14].

Our results are similar to another study done on patients with follicular variant PTC, which reported that hypoechogenicity, microcalcifications, and irregular margins were found more frequently with BRAF positive follicular variant PTC [21]. The study concluded that more suspicious US features of thyroid nodules were found in the BRAF positive group (88.6 vs. 57.1%, $p = 0.002$) [21], while another study that included 137 patients with follicular variant PTC found that hypoechogenicity and irregular margins were significantly associated with BRAF positive tumors [19]. Other studies found that BRAF positive tumors were smaller than BRAF negative ones [13, 14, 19]; however, we found that the nodule size was comparable between BRAF positive and negative cases. They attributed the smaller size of BRAF positive tumors to the suspicious US features that hasten early intervention [19].

Most of the studies that assessed the association between BRAFV600E mutation and suspicious US features in PTC found an association between some of these features and BRAF positivity, but there was no consensus around the suspicious features that predict BRAF status. In our experience, calcifications and irregular margins on US showed the highest likelihood ratio for BRAF positive tumors; however, hypoechogenicity, LNM, and ETE were also more frequent in BRAF positive cases. Further large-scale studies are needed to investigate this association and to determine how this information can be used to better plan the management of PTC patients.

Limitations of our sturdy include its retrospective nature and the use of cervical US, which is operator dependent. However, all cervical US examinations were done by the same endocrine surgeon and the same trained US technologist, so the inter-observer variability in interpreting nodular characteristics is not a concern in our study.

Conclusion

BRAF positive PTC was associated with a higher rate of LN metastasis and the rate of ETE to BRAF negative PTC. The presence of multiple suspicious US findings of thyroid nodules can predict the BRAFV600E mutation status of PTC nodules. The presence of hypoechogenicity, intra-nodular calcification and irregular nodular margins were the most predictive features of BRAFV600E positivity. Suspicious LNs detected by US could not predict BRAF status of PTC. Future multi-institutional studies are warranted to assist surgeons in risk stratification and surgery planning for patients with PTC.

Disclosure Statement

The authors whose names are listed above certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or nonfinancial interest in the subject matter or materials discussed in this manuscript.

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