Evaluation of Parallel Diagnostic Test Combining BRAF V600E Mutation with TI-RADS on Thyroid Nodules

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Abstract

Aim: To evaluate the parallel test combining BRAF V600E mutation test with thyroid imaging reporting and data system (TI-RADS) for diagnosis of malignant thyroid nodules on Chinese patients.

Methods: A retrospective study of 131 consecutive patients with thyroid nodule diseases who underwent thyroid ultrasonography and BRAF V600E mutation tests was conducted in Xiamen, China. For the parallel test, nodules which were considered as malignant by either the TI-RADS classifications or the BRAF V600E mutation tests were all diagnosed as malignant.

Results: Of the 131 nodules, 44 (33.6%) were diagnosed as malignant, and malignant nodules were significantly more likely to be hypoechoic, irregular in shape, edge disjoint on edge, unclear along boundaries, micro calcifications and companied with abnormal lymph nodes when compared with benign lymph nodes. The proportions of higher TI-RADS grades and positive BRAF V600E mutation were significantly increased in malignant nodules than benign nodules (both p-values <0.05). Area under the curves with 95% confidence interval of receiver operating characteristic curves were 0.798 (0.719-0.877; p<0.001) for the TI-RADS classification, 0.846 (0.764-0.928; p<0.001) for BRAF V600E mutation, and 0.810 (0.738-0.883; p<0.001) for the parallel test, but differences on them were not statistically significant. Compared with the TI-RADS classification system and BRAF V600E mutation test alone, parallel test showed significantly higher sensitivity and negative predictive value; BRAF V600E mutation test showed significantly higher specificity and positive predictive value than the other two methods.

Conclusion: TI-RADS classification and BRAF V600E mutation test are both effective methods for diagnosis of malignant thyroid nodules, and parallel test combining these two methods improves sensitivity and negative predictive value.

Keywords: TI-RADS; BRAF V600E; Parallel test; Thyroid nodules

Abbreviations

AUC: Area under Curve; PTC: Papillary Thyroid Carcinoma; ROC: Receiver Operating Characteristic; TI-RADS: Thyroid Imaging Reporting and Data system

Introduction

Thyroid nodules are common findings during ultrasound imaging examination of the thyroid. Although only 5% to 15% of thyroid nodules are malignant, the incidence of thyroid cancer continues to rise [1]. Ultrasonography remains the most important method for the initial evaluation of thyroid nodules since it provides valuable reference for the detection and diagnoses of potentially malignant thyroid nodules [2]. Thyroid imaging reporting and data system (TI-RADS) classifies thyroid nodules on the basis of conventional ultrasound imaging and provides useful guidance during selection of the treatment approach [3,4]. Although several different TI-RADS systems have been proposed to standardize thyroid ultrasound reports, none of them has been uniquely accepted [5,6].

Papillary Thyroid Carcinoma (PTC) is the most common type of thyroid cancer [7]. The BRAF gene has a high mutation rate of around 29% to 83% for PTC [8,9]. BRAF V600E mutation, which is a representative genetic mutation in the BRAF gene, is reported to lead to abnormal activation of the

Mitogen-Activated Protein Kinase (MAPK) pathway and thus plays a crucial role in the initiation and progression of PTC [10,11]. PTC with BRAF V600E mutation shows aggressive behavior, tending to invade surrounding thyroid tissue and spread to lymph nodes [8,10-11]. The presence of BRAF V600E mutation, even highly differentiated papillary carcinoma, may develop into poorly differentiated or undifferentiated cancer [12,13]. Thus, BRAF V600E gene mutation detection could serve to identify PTC patients with poor prognosis. Although BRAF V600E mutation has been frequently reported to be associated with PTC, few studies have been evaluated the diagnostic value of BRAF V600E mutation, especially in addition to TI-RADS, on thyroid nodule diseases.

The aim of the present study was to evaluate the parallel test by combining BRAF V600E mutation detection with TI-RADS on malignant thyroid nodule, with the histopathological results of pathological examination as the gold standard, on 131 Chinese patients with thyroid nodule diseases.

Materials and Methods

Patients

A total of 131 consecutive patients with thyroid nodule diseases (32 males and 99 females; age range: 8 to 78 years; age mean: 42.3 (± 14.6) years) who underwent ultrasonography and pathological examinations at the First Affiliated Hospital of Xiamen University (China) between September 2015 and November 2017 were enrolled in this retrospective study. Data on age, sex and pathological examinations were collected from their medical records. Histopathological results of pathological examination on all 131 nodules were obtained. The study was conducted in accordance with the Helsinki Declaration and the International Conference on Harmonization/Good Clinical Practice guidelines. The study protocol was approved by the human ethics committees of the First Affiliated Hospital of Xiamen University, Xiamen, China. Written informed consent was obtained from all patients.

Ultrasound examination

Ultrasound scanning was performed on each patient using Philips iu22, Philips EPIQ7 (Boston, USA) or GE LOGIC E9 (Milwaukee, USA) scanners equipped with 5 MHz to 13 MHz linear array probes. The patient was positioned supine, without a pillow. The thyroid gland was thoroughly scanned first, and then the neck lymph nodes were examined one by one. The instrument's preset thyroid mode was used for a preliminary scan, and the gain, depth, focus, harmonics, and other parameters were then adjusted as necessary to obtain the best image quality for each lesion. Nodule size, morphology, and cervical lymph node status were recorded.

TI-RADS classification

A modified TI-RADS systems were used to classify thyroid nodules into six categories: 1: Normal thyroid gland; 2: Cystic, solid-cystic, or spongy nodule accompanied by glial formation; 3: Cystic nodule without glial formation, or isoechoic or hyperechoic solid nodules; 4 (three subcategories): 4a: Nodules with one of these four features of malignancy: solid hypoechoic or very hypoechoic nodule, micro calcification (including mixed calcifications), irregular edges (including blurred, angulated or lobulated borders), and aspect ratio >1; 4b: Nodules displaying any two of the four features mentioned above; 4c: Nodules displaying any three of the four features mentioned above; 5: Nodule displaying all four of the features mentioned in Category 4, or protruding outside the thyroid capsule or invading

surrounding tissue, or accompanied by structural abnormalities in the cervical lymph nodes; 6: Malignant thyroid nodules with diagnosis confirmed by diagnostic puncture or surgical pathology [3].

Ultrasound-guided thyroid puncture biopsy

Informed consent was obtained before puncture, and the patient was routinely prepared for the procedure. The puncture was performed using a 20G needle, with the site of puncture, angle, and depth of insertion of the needle determined by the location of the nodule. During puncture the aim was to obtain tissue containing micro calcification (if present) and, for cystic-solid nodules, to obtain specimen from the solid part of the nodule or the wall of the cyst. The aspirated specimen was pushed out evenly onto glass slides and sent for the pathological examination. A specimen was also sent for BRAF V600E gene detection.

BRAF V600E mutation detection

DNA was extracted from specimens using a commercial DNA isolation kit (QIAamp DNA FFPE Tissue Kit; Qiagen, Beijing, China) according to the manufacturer's protocol. DNA was diluted with distilled water to a concentration of approximately 2 ng/μl - 3 ng/μl. Mutations at the BRAF V600E site were detected using a human BRAF V600E ARMS-PCR kit (Aide Biotechnology Co., Ltd. gene, Xiamen, China). The quality of the extracted DNA was verified by the amplification of a housekeeping gene reported in the HEX channel. Amplification was performed on an ABI Prism 7500 thermo cycler (Life Technologies, Carlsbad, California, USA) according to the manufacturer's protocol. The run files were analyzed and interpreted in accordance with the manufacturer's instructions specified.

Parallel test combining TI-RADS classification with BRAF V600E mutation

Each nodule was separately diagnosed by using both TI-RADS classification and the BRAF V600E mutation test. For the BRAF V600E mutation test separately, nodules were considered as malignant for the positive tests and as benign for the negative tests. For the TI-RADS classification, category 4b or higher were considered as malignant and category 4a or lower were considered benign. For the diagnosis of parallel test by combining the TI-RADS classification with BRAF V600E mutation, nodules which were considered as malignant by either the TI-RADS classifications or the BRAF V600E mutation tests were all diagnosed as malignant, and only nodules which were considered as benign by both the TI-RADS classifications and the BRAF V600E mutation tests were diagnosed as benign. Diagnostic value of each method was evaluated by regarding the pathological examination results as the gold standards.

Statistical analysis

Data was presented as the mean ± standard deviation for continuous variable or number and percentage for categorical variable. Differences between patients categorized as benign or malignant according to the results of pathological examination were analyzed using one-way ANOVA for continuous variables and chi-square test for categorical variables. Receiver Operating Characteristic (ROC) curves were constructed for each diagnosis method (the TI-RADS classification system, BRAF V600E mutation test, and the parallel test combining TI-RADS classification system with BRAF V600E mutation test), and the Area under the Curves (AUCs) were calculated for all the three diagnosis methods and compared among them. Chi-square tests were used to compare the sensitivities, specificities, positive predictive values, and negative predictive value

Table 1: Differences of clinical and ultrasound features between malignant and benign thyroid nodules.

Variables	Benign nodules	Malignant nodules	Total	P value
N (%)	87 (66.4%)	44 (33.6%)	131 (100.0%)	
Age (years)	42.7 ± 14.9	41.5 ± 13.8	42.3 ± 14.5	0.653
Sex				0.087
Female (n, %)	69 (79.3%)	29 (65.9%)	98 (74.8%)	
Male (n, %)	18 (20.7%)	15 (34.1%)	33 (25.2%)	
Echogenicity				**<0.001**
Solid–cystic	31 (35.6%)	3 (6.8%)	34 (26.0%)	
Low echo	54 (62.1%)	40 (90.9%)	94 (71.8%)	
High echo	2 (2.3%)	1 (2.3%)	3 (2.3%)	
Shape				0.01
Oval or round	72 (82.8%)	27 (61.4%)	99 (75.6%)	
Irregular	15 (17.2%)	17 (38.6%)	32 (24.4%)	
Capsule contact				<0.001
Yes	79 (90.8%)	24 (54.5%)	103 (78.6%)	
No	8 (9.2%)	20 (45.5%)	28 (21.4%)	
Margin				<0.001
Well-defined	77 (88.5%)	27 (61.4%)	104 (79.4%)	
Ill-defined	10 (11.5%)	17 (38.6%)	27 (20.6%)	
Calcification				<0.001
No-calcification	60 (69.0%)	13 (29.5%)	73 (55.7%)	
Micro-calcification	20 (23.0%)	29 (65.9%)	49 (37.4%)	
Macro-calcification	7 (8.0%)	2 (4.5%)	9 (6.9%)	
Ratio of height and width				0.803
>1	7 (8.0%)	3 (6.8%)	10 (7.6%)	
≤ 1	80 (92.0%)	41 (93.2%)	121 (92.4%)	
Abnormal lymph nodes				0.003
Yes	2 (2.3%)	8 (18.2%)	10 (7.6%)	
No	85 (97.7%)	36 (81.8%)	121 (92.4%)	
Optimized TI-RADS classification				<0.001
Category 3	32 (36.8%)	2 (4.5%)	34 (26.0%)	
Category 4a	26 (29.9%)	6 (13.6%)	32 (24.4%)	
Category 4b	17 (19.5%)	13 (29.5%)	30 (22.9%)	
Category 4c	9 (10.3%)	13 (29.5%)	22 (16.8%)	
Category 5	3 (3.4%)	10 (22.7%)	13 (9.9%)	
BRAF V600E mutation				<0.001
Yes	5 (5.7%)	33 (75.0%)	38 (29.0%)	
No	82 (94.3%)	11 (25.0%)	93 (71.0%)	

All percentages are column percentage

among the three diagnosis methods. All p-values were two-sided and p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS 19.0 (IBM Corp., Armonk, NY, USA) and R 3.5.0 (http://www.R-project.org/).

Results

### Clinical characteristics stratified by malignant thyroid nodule

Of the 131 nodules, 44 (33.6%) were diagnosed as malignant and 87 (66.4%) were benign based on the histopathological results of pathological examination or biopsy. The maximum diameter of

the nodules ranged from 0.4 cm to 6.9 cm. Table 1 shows the clinical differences of all thyroid nodules stratified by malignant nodules. There was no significant difference on age and gender between the malignant and benign groups. For ultrasonography examinations, malignant nodules were significantly more likely to be hypoechoic, irregular in shape, edge disjoint on edge, unclear along boundaries and micro calcifications when compared with benign lymph nodes. Furthermore, malignant thyroid nodules were also significantly more likely companied with abnormal lymph nodes than those controls. The proportion of higher TI-RADS grades were significantly increased for malignant nodules than benign nodules (p-value <0.001). The

Table 2: Comparison of diagnostic values of three methods for diagnosis of malignant thyroid nodules.

	TI-RADS classification	BRAF V600E mutation	Parallel test	P value		
Indices	-1	-2	-3	(1) *vs.* (2)	(2) *vs.* (3)	(1) *vs.* (3)
AUC of ROC	0.798	0.846	0.81	0.128	0.382	0.166
Sensitivity	0.818	0.75	0.977	0.604	0.005*	0.035*
Specificity	0.667	0.943	0.644	<0.001*	<0.001*	0.873
Positive predictive value	0.554	0.868	0.581	0.002*	0.004*	0.879
Negative predictive value	0.879	0.882	0.982	1	0.031*	0.037*

*p<0.05

Abbreviations: AUC: Area Under Curve; ROC: Receiver Operating Characteristic; TI-RADS: Thyroid Imaging Reporting and Data System
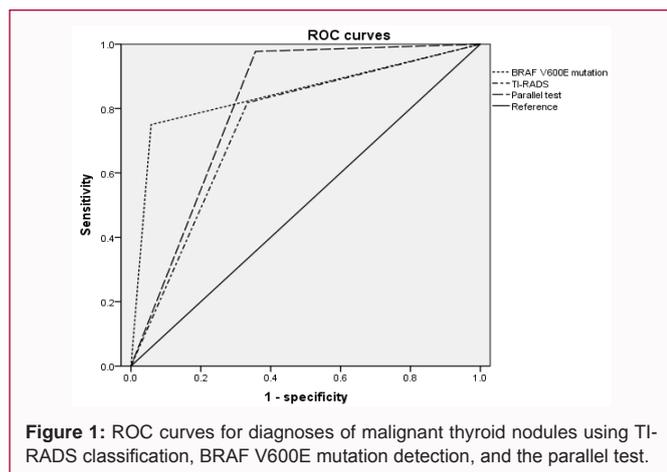
Figure 1: ROC curves for diagnoses of malignant thyroid nodules using TI-RADS classification, BRAF V600E mutation detection, and the parallel test.

proportion of positive BRAF V600E mutation was significantly higher in malignant nodules (33/44, 75.0%) than those in benign nodules (5/87, 5.7%) (p-value <0.001). And the likelihood of *BRAF* V600E mutation increased significantly with the increasing TI-RADS grades (Kruskal–Wallis test, chi-square value: 17.238; p-value <0.05).

ROC curves for diagnosis of malignant thyroid nodule

Figure 1 shows the ROC curves of malignant thyroid nodule diagnoses by using three methods (the TI-RADS classification system, BRAF V600E mutation test, and the parallel test combining these two methods). The Area under the Curve (AUC) with 95% Confidence Interval (CI) were 0.798 (0.719 to 0.877; p-value <0.001) for the TI-RADS classification, 0.846 (0.764 to 0.928; p-value <0.001) for BRAF V600E mutation, and 0.810 (0.738 to 0.883; p-value <0.001) for the parallel test, respectively. The differences of AUC for the three diagnosis methods were not statistically significant (all p-values >0.05, Table 2).

Diagnosis values of parallel test combining BRAF V600E mutation with TI-RADS on malignant thyroid nodules

Table 2 shows the indices of diagnostic values on malignant thyroid nodules by using three different methods. The sensitivities, specificities, positive predictive values, and negative predictive values of the three methods for diagnosis of malignant thyroid nodule were as follows: 0.818, 0.667, 0.554 and 0.879 for TI-RADS classification, respectively; 0.750, 0.943, 0.868, and 0.882 for BRAF V600E mutation, respectively; and 0.977, 0.644, 0.581 and 0.982 for the parallel test combining the two methods, respectively (Table 2). Compared with the TI-RADS classification system and BRAF V600E mutation test alone, parallel test showed significantly higher sensitivity and negative predictive value. But BRAF V600E mutation test showed significantly higher specificity and positive predictive value than the TI-RADS

classification system alone and the parallel test.

Discussion

The present study provided for the first time the evaluation of the parallel diagnostic test combining the TI-RADS classification system with BRAF V600E mutation detection on malignant thyroid nodules. We found that, compared with the TI-RADS classification system and BRAF V600E mutation test separately, parallel test showed significantly higher sensitivity and negative predictive value, although there was no significant difference on AUC of ROC curves for these three diagnostic methods. Furthermore, BRAF V600E mutation test showed significantly higher specificity and positive predictive value than the TI-RADS classification system and the parallel test.

Several TI-RADS classification systems have been proposed to standardize thyroid ultrasound reports [3-6], but it has little been evaluated on Chinese patients with thyroid nodules. The present study found that malignant thyroid nodules showed significantly higher TI-RADS grades, which was consistent with the reports from Moon et al. and others [14,15]. BRAF V600E mutation, an activating point mutation of the T1799A point BRAF gene, results in a valine-to-glutamic acid replacement at amino acid V600 and induces the tumorogenesis [16-18]. Our study consistently showed a significantly high proportion of BRAF V600E mutation in malignant nodules than in benign nodules. And we also found a significant association between BRAF V600E mutation and the TI-RADS classification grades, with the frequencies of positive BRAF V600E mutation elevating with the TI-RADS grades increasing.

Although BRAF V600E mutation has been consistently shown to be associated with PTC, few studies evaluated the diagnosis values of BRAF V600E mutation for Chinese patients with thyroid nodules, and none has been further performed to evaluate the parallel diagnostic test combining BRAF V600E mutation test with the TI-RADS classification system. The present study found that, although there was no significant difference on AUC of ROC curves for the TI-RADS classification, BRAF V600E mutation, and the parallel test combining these two methods, the parallel test did show significantly higher sensitivity and negative predictive value than other two methods alone. Furthermore, BRAF V600E mutation test showed significantly higher specificity and positive predictive value than the TI-RADS classification system and the parallel test.

Based on findings of the present study, we would suggest as follows. First, TI-RADS classification system, BRAF V600E mutation, and the parallel test combining these two methods were all effective diagnostic methods and showed similar diagnostic accuracies, since AUC of ROC curves for them were relatively high and comparable. Furthermore, BRAF V600E mutation test showed significantly

higher specificity and positive predictive value and the parallel test showed significantly higher sensitivity and negative predictive value. Therefore, for thyroid nodules with TI-RADS categories of 4b or above, BRAF V600E mutation test and parallel test should be performed to improve specificity and sensitivity accordingly. Second, some nodules with TI-RADS category 3 may have certain degrees of malignancy. Therefore, these patients should be closely followed up. Third, because mutation on BRAF V600E is associated with malignant behavior and metastasis [8,10,11], identification of this mutation can be valuable for prognosis and guiding treatment decisions.

A few limitations in the present study should be acknowledged. First, our study was a retrospective study and bias on information collection was inevitable. For example, ultrasound imaging data stored was not reliable like real-time imaging data, but most previous researches also had this limitation [19]. Second, the sample size of the present study was relatively small. Therefore, an independent cohort with larger sample size should be conducted to validate our findings in future. Third, although observers' judgment of ultrasound imaging data was based on clinical data and normative guidance experience, this judgment is inevitably objective.

In conclusion, TI-RADS classification system and BRAF V600E mutation detection are both effective methods for the diagnosis of malignant thyroid nodules. And the parallel test combining these two methods improves sensitivity and negative predictive value.

Author Contribution

GS and ZL participated in the design of the study and revised the manuscript; JS, CZ and CF participated in the data collection; JS and ZL performed the statistical analysis and draft the manuscript. All authors have read and approved the final manuscript. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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References

1. Cantisani V, Maceroni P, D'Andrea V, Patrizi G, Di Segni M, De Vito C, et al. Strain ratio ultrasound elastography increases the accuracy of colour-Doppler ultrasound in the evaluation of Thy-3 nodules. A bi-centre university experience. Eur Radiol. 2016; 26(5):1441-9.

2. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 american thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the american thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26(1):1-133.

3. Horvath E, Majlis S, Rossi R, Franco C, Niedmann JP, Castro A, et al. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab. 2009;94(5):1748-51.

4. Na DG, Baek JH, Sung JY, Kim JH, Kim JK, Choi YJ, et al. Thyroid Imaging Reporting and Data System Risk Stratification of Thyroid Nodules: Categorization Based on Solidity and Echogenicity. Thyroid. 2016;26(4): 562-72.

5. Maia FF, Matos PS, Pavin, EJ, Zantut-Wittmann DE. Thyroid imaging reporting and data system score combined with Bethesda system for malignancy risk stratification in thyroid nodules with indeterminate results on cytology. Clin Endocrinol (Oxf). 2015;82(3):439-44.

6. Grant EG, Tessler FN, Hoang JK, Langer JE, Beland MD, Berland LL, et al. Thyroid ultrasound reporting lexicon: white paper of the acr thyroid imaging, reporting and data system (TIRADS) committee. J Am Coll Radiol. 2015;12(12 Pt A):1272-9.

7. La Vecchia C, Malvezzi M, Bosetti C, Garavello W, Bertuccio P, Levi F, et al. Thyroid cancer mortality and incidence: a global overview. Int J Cancer. 2015;136:2187-95.

8. Xing M. BRAF mutation in thyroid cancer. Endocrine Related Cancer. 2005;12(2):245.

9. Kebebew E, Weng J, Bauer J, Ranvier G, Clark OH, Duh QY, et al. The prevalence and prognostic value of BRAF mutation in thyroid cancer. Ann Surg. 2007;246(3):466-70.

10. Koh J, Choi JR, Han KH, Kim EK, Yoon JH, Moon HJ, et al. Proper indication of BRAF(V600E) mutation testing in fine-needle aspirates of thyroid nodules. PLos One. 2013;8(5):e64505.

11. Kwak JY, Kim EK, Chung WY, Moon HJ, Kim MJ, Choi JR. Association of BRAFV600E mutation with poor clinical prognostic factors and US features in Korean patients with papillary thyroid microcarcinoma. Radiology. 2009;253(3):854-60.

12. Hilger RA, Scheulen ME, Strumberg D. The Ras-Raf-MEK-ERK pathway in the treatment of cancer. Onkologie. 2002 ;25(6):511-8.

13. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin Endocrinol Metab. 2011;96(11): 3390-7.

14. Moon WJ, Choi N, Choi JW, Kim SK, Hwang TS. BRAF mutation analysis and sonography as adjuncts to fine-needle aspiration cytology of papillary thyroid carcinoma: their relationships and roles. AJR Am J Roentgenol. 2012; 198:668-74.

15. Kwak JY, Han KH, Yoon JH, Moon HJ, Son EJ, Park SH, et al. Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. Radiology. 2011;260(3): 892-9.

16. Xing M. BRAF mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. Endocr Rev. 2007;28:742-62.

17. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol. 2011;7(10):569-80.

18. Hwang J, Shin JH, Han BK, Ko EY, Kang SS, Kim JW, et al. Papillary thyroid carcinoma with BRAFV600E mutation: sonographic prediction. Am J Roentgenol. 2010;194:W425-30.

19. Xie C, Cox P, Taylor N, LaPorte S. Ultrasonography of thyroid nodules: a pictorial review. Insights Imaging. 2016;7(1):77-86.