



# ***BRAF*<sup>V600E</sup> mutation combined with American College of Radiology thyroid imaging report and data system significantly changes surgical resection rate and risk of malignancy in thyroid cytopathology practice**

Yun Zhu<sup>1</sup>, Hongxun Wu<sup>2</sup>, Botao Huang<sup>1</sup>, Xin Shen<sup>1</sup>, Gangming Cai<sup>3</sup>, Xiaobo Gu<sup>3</sup>

<sup>1</sup>Department of Pathology, Jiangyuan Hospital Affiliated to Jiangsu Institute of Nuclear Medicine, Wuxi, China; <sup>2</sup>Department of Ultrasound, Jiangyuan Hospital Affiliated to Jiangsu Institute of Nuclear Medicine, Wuxi, China; <sup>3</sup>Clinical Molecular Biology Laboratory, Jiangyuan Hospital Affiliated to Jiangsu Institute of Nuclear Medicine, Wuxi, China

**Contributions:** (I) Conception and design: Y Zhu; (II) Administrative support: Y Zhu, H Wu; (III) Provision of study materials or patients: Y Zhu, H Wu, G Cai; (IV) Collection and assembly of data: Y Zhu, B Huang, G Cai; (V) Data analysis and interpretation: Y Zhu, X Shen; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

**Correspondence to:** Yun Zhu, MD. Department of Pathology, Jiangyuan Hospital Affiliated to Jiangsu Institute of Nuclear Medicine, No. 20 Qianrong Road, Binhu District, Wuxi, China. Email: zhuyun@jsinm.org.

**Background:** Ultrasonography patterns and molecular testing may assist in stratifying the malignancy risk of indeterminate cytology diagnosis. The purpose of this study was to assess the value of fine needle aspiration (FNA) cytology in combination with American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS) and *BRAF*<sup>V600E</sup> mutation in differentiating high-risk thyroid nodules.

**Methods:** From February 2010 to February 2014, 2,643 consecutive thyroid nodules from 2,399 patients (688 men and 1,711 women; mean age, 44.3±12.5 years) who underwent preoperative FNA biopsies were enrolled. The Jiangsu Institute of Nuclear Medicine has adopted TI-RADS stratification and *BRAF*<sup>V600E</sup> mutation analysis as a routine procedure to assist in evaluating FNA cytopathology since January 2016. From February 2017 to July 2018, 1,905 thyroid nodules of 1,837 patients (501 men and 1,336 women, 49.5±12.8 years) who underwent preoperative ultrasound-guided FNA biopsies with *BRAF*<sup>V600E</sup> mutation analysis and ACR TI-RADS grading data available were enrolled for comparison in this study.

**Results:** The cancer risk in nodules with *BRAF*<sup>V600E</sup> mutation was 99.7% (905/908) according to the histological findings. The risk of malignancy (ROM) was found to increase with advancing ACR TI-RADS category. High-risk ultrasound features (TI-RADS 5) did show a good performance in predicting malignancy (98.1%). The combination of TI-RADS 5 and *BRAF*<sup>V600E</sup> mutation reached the best diagnostic efficiency [sensitivity 97.7%, specificity 94.8%, positive predictive value (PPV) 99.6%]. It is apparent that after the implementation of ACR TI-RADS and the *BRAF*<sup>V600E</sup> mutation analysis, the resection rates (RRs) of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) I and III categories showed significant decreases (39.1% vs. 21.6% and 36.1% vs. 16.7%, respectively). In contrast, the risks of malignancy of TBSRTC I and III categories indicated substantial increases (41.5% vs. 80.0% and 34.6% vs. 50.0%, respectively). The ROM of thyroid nodules with nondiagnostic (ND, I) category showed the most significant increase of 41.5% to 80.0%.

**Conclusions:** ACR TI-RADS, together with *BRAF*<sup>V600E</sup> mutation and cytological diagnoses, can assist in improving the prediction of malignancy of thyroid nodules, especially in the TBSRTC I and III categories.

**Keywords:** American College of Radiology Thyroid Imaging Reporting and Data System (ACR TI-RADS); *BRAF*<sup>V600E</sup> mutation; fine needle aspiration (FNA); risk of malignancy (ROM)

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## Introduction

Fine needle aspiration (FNA) biopsy of the thyroid gland has been developed as a primary procedure for evaluating patients with thyroid nodules (1). Ultrasonography is recommended as an essential method in malignancy risk stratification for thyroid nodules and for decisions concerning FNA of thyroid nodules (2). The American College of Radiology (ACR) has created an ultrasound malignancy risk stratification system for thyroid nodules named the ACR Thyroid Imaging Reporting and Data System (TI-RADS), in which the malignancy risk is stratified by suspicious ultrasound features (3). The ACR TI-RADS has demonstrated some capacity to select high risk-patients for FNA biopsy, and a combination of cytopathology and TI-RADS has shown a considerable ability to differentiate high-risk thyroid nodules (4,5).

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was developed by the American National Cancer Institute in 2009 to standardize the interpretation of the FNA cytology (FNAC) results (6). The TBSRTC system has been widely used and helps reduce unnecessary surgeries (7). A few specific output measurements, including frequency of thyroid FNA samples, resection rate (RR), and risk of malignancy (ROM) in each diagnostic category, serve as quality control criteria (1,6).

The utility of molecular techniques for thyroid nodules has wide application, and it may enhance the diagnostic value of FNA (8).  $BRAF^{V600E}$  is the most specific single molecular diagnostic test for thyroid lesions (9), and all thyroid tumors which contain  $BRAF^{V600E}$  mutation are considered malignant (10).

In this study, we evaluated the diagnostic value of ACR TI-RADS and  $BRAF^{V600E}$  mutation analysis based on the corresponding FNAC and surgical histopathology results. We calculated the RR and ROM for TBSRTC before and after the implementation of ACR TI-RADS and  $BRAF^{V600E}$  mutation analysis at our institute. This is a validation study of the diagnostic performance of ACR TI-RADS, FNAC, and  $BRAF^{V600E}$  molecular test analysis in a Chinese population. We present the following article in accordance with the STARD reporting checklist (available at [http://](http://dx.doi.org/10.21037/gS-20-535)

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## Methods

### Subjects

This was a retrospective study. The study protocol was reviewed and approved by the Institutional Review Board of Jiangsu Institute of Nuclear Medicine (IRB No. YL201601) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Informed consent was obtained from all patients included in the study. Incidental microcarcinomas detected in surgical specimens were excluded from statistical analysis.

From February 1, 2010, to February 1, 2014, a total of 2,643 consecutive thyroid nodules from 2,399 patients (688 men and 1,711 women) underwent preoperative ultrasound-guided or palpation-guided FNA biopsies. Thyroid ultrasound records were available for all patients who were included in this study. During this period, all thyroid nodules did not undergo ACR TI-RADS stratification and the  $BRAF^{V600E}$  mutation test. The average age was  $44.3 \pm 12.5$  years old, and the mean diameter was  $1.4 \pm 0.9$  cm (Table 1). The cytological reports were adjusted with the 2009 TBSRTC system and classified into six categories. Our institution has used the TI-RADS stratification and  $BRAF^{V600E}$  mutation analysis as a routine procedure since January 2016. Between February 1, 2017, and July 31, 2018, a total of 2,167 consecutive thyroid nodules were found from 2,011 patients who underwent preoperative ultrasound-guided FNA biopsies. During this period, nodules that met the following criteria were included in this survey: (I) thyroid cytology based on the six-tiered 2009 TBSRTC system; (II) availability of  $BRAF^{V600E}$  mutation analysis; (III) an ultrasound examination of the nodules classified by the five-tiered ACR TI-RADS. Finally, a total of 1,905 thyroid nodules from 1,837 patients (501 men and 1,336 women) were enrolled; 262 out of 2,167 nodules were excluded from the survey because patients refused to undergo molecular tests, or the ultrasound contained only a descriptive report without ACR TI-RADS grading. The average age was  $49.5 \pm 12.8$  years old, and the mean diameter was  $1.1 \pm$

**Table 1** Clinical data of the study population

Features	2010.02–2014.02	2017.02–2018.07	P value
Number of patients	2,399	1,837	
Number of nodules	2,643	1,905	
Age (years), mean ± SD	44.3±12.5	49.5±12.8	0.000
Nodule size (cm), mean ± SD	1.4±0.9	1.1±0.7	0.000
Male: female	688:1,711	501:1,336	

0.7 cm. The difference in average age and nodule diameter between the periods spanning 2010 to 2014 and 2017 to 2018 was significant ( $P < 0.01$ ) (Table 1).

Three cytopathologists specializing in thyroid pathology shared the work, and each sample was assessed by two cytopathologists. Our institution has adopted the original TBSRTC system to reassess the cytological reports. This system uses the following six-tiers for risk categorization (6): nondiagnostic (ND, I), benign (II), atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS, III), follicular neoplasm/suspicious for follicular neoplasm (FN/SFN, IV), suspicious for malignancy (SFM, V); and malignant (VI) (1). Our institution has adopted the borderline tumor category introduced by the latest World Health Organization classification of endocrine organs (11) in routine histopathology practice, which includes follicular tumor of uncertain malignant potential (FT-UMP), well-differentiated tumor of uncertain malignant potential (WDT-UMP), and non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP).

### Criteria for FNA

The FNA criteria mainly included enlarging nodule size, abnormal serological tests, suspicious ultrasound features, and clinician preference. After the adoption of ACR TI-RADS, ultrasound evaluation as TI-RADS 5 was recommended for FNA biopsy. FNA is not recommended for TI-RADS 1 and TI-RADS 2 thyroid nodules because of the low potential for malignancy (3). TI-RADS 3 nodules measuring  $\geq 2.5$  cm and TI-RADS 4 nodules measuring  $\geq 1.5$  cm were recommended for FNA biopsy (12), other factors indicating FNA included patients who had been exposed to previous radiation to the neck, those with a family history of thyroid cancer, and those with nodule growth observed during surveillance (8).

### Criteria for surgical treatment

Thyroid surgery was recommended to all FNA-*BRAF*<sup>V600E</sup> mutation-positive patients regardless of the cytological reports. For *BRAF*<sup>V600E</sup> mutation-negative cases, patients with SFM (V) or malignant (VI) were recommended for surgeries. Repeated ultrasound-guided FNA or follow-up ultrasound was recommended for the patients with ND (I) and AUS/FLUS (III) reports. FN/SFN (IV) with a TI-RADS 4 or TI-RADS 5 ultrasound report would be recommended for surgical treatment, otherwise those with a TI-RADS 2 or TI-RADS 3 score were recommended to undergo active surveillance. Patients with benign (II) reports were commonly recommended for ultrasound follow-up except for those with hyperfunctioning goiter, pressure symptoms, or if the enlarged nodule would influence appearance.

### Ultrasonography examination

Ultrasound examinations and ACR TI-RADS were conducted as described previously (13). TI-RADS, consisting of a five-tiered risk classification based on suspicious ultrasound features [2,3], has been used to classify each thyroid nodule at our institution since March 2015. All ultrasound examinations were performed using a 5–12 MHz linear-array probe (iU22, Philips Healthcare, Bothell, WA, USA) by 1 of 10 radiologists who had 3 to 24 years of experience in thyroid imaging (13).

### FNA procedure and *BRAF*<sup>V600E</sup> detection

Ultrasound-guided FNA was performed for nodules with suspicious features by 1 of 5 certified radiologists, using a previously described procedure (13,14). The procedure was conducted in the hospital outpatient setting after informed consent was obtained from the patient. Thyroid FNA samples were collected under ultrasound guidance

with a 23-gauge needle attached to a 5-mL syringe. Two to four passes were made for each thyroid nodule. The slides were wet-fixed in 95% ethanol and then sent for hematoxylin and eosin staining. On-site cytological assessment was not available. The residual biopsy samples in the syringe were expelled for molecular testing after cytological preparation. The wash-out of the needle tip was pipetted into a microcentrifuge tube for molecular testing. DNA was extracted using the QIAamp DNA Micro kit (Qiagen) according to the manufacturer's instructions. The method for *BRAF*<sup>V600E</sup> mutation analysis has been described previously (13,15).

### Statistical analysis

Statistical analysis was performed with SPSS statistical software, version 23.0 (IBM, Armonk, NY, USA). Two-tailed chi-square ( $\chi^2$ ) test or Fisher's exact test was used to evaluate potential differences in the categorical variables between groups. Two-sample *t*-test was adopted for the comparison of variations. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy (AC) of ACR TI-RADS, *BRAF*<sup>V600E</sup> mutation, and the combination of the two methods were evaluated. For all tests, a P value <0.05 was considered an indication of a significant difference.

## Results

### Test for *BRAF*<sup>V600E</sup> mutation

As shown in *Figure 1*, The percentages of *BRAF*<sup>V600E</sup> mutation rates in 1,905 FNA samples, according to the TBSRTC I–VI categories, were 12.9%, 1.3%, 5.6%, 2.6%, 79.8%, and 82.2%, respectively. A total of 1,036 nodules out of 1,905 had final histological diagnoses, and 978 were found to be malignant. Of the 1,036 surgical-treated specimens, borderline tumors accounted for a minimal proportion in each TBSRTC category, with none being ND (I) or benign (II), two (2/18) being AUS/FLUS (III), five (5/40) being FN/SFN (IV), one (1/75) being SFM (V), and two being (2/861) malignant (VI) (*Figure 1*). The 25 nodules with TBSRTC I–III cytology reports and positive mutation results were recommended for surgical treatment. One patient refused surgery, and 24 nodules proved to be papillary thyroid carcinomas (PTCs). Another 504 nodules classified as TBSRTC I–III cytology reports, and negative for the *BRAF*<sup>V600E</sup> mutation, were recommended

for surveillance every 6 months using ultrasound with or without repeated FNA that did not exhibit any changes over time.

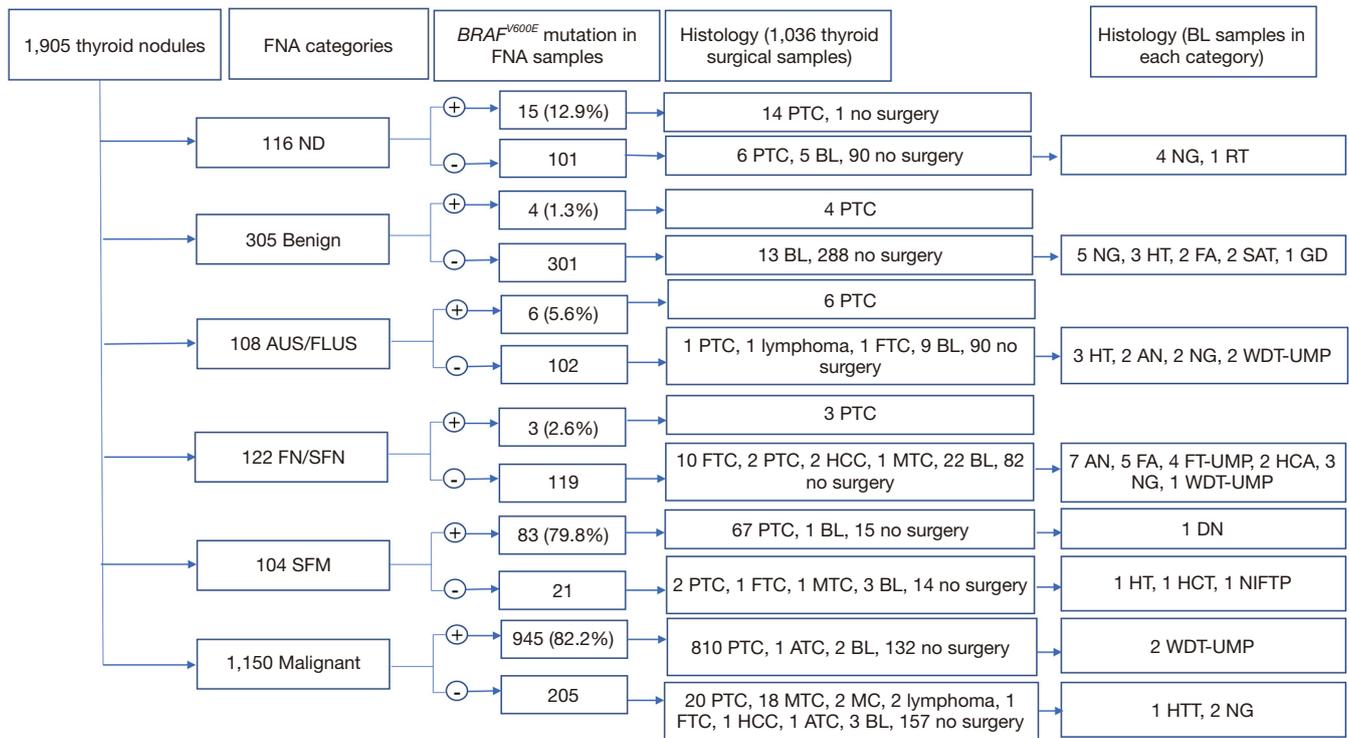
*BRAF*<sup>V600E</sup> mutation was found in 1,028 nodules with SFM (V) or malignant (VI) cytology. In all, 881 of these nodules had histopathological results, including 877 PTCs, one anaplastic carcinoma, and three benign/borderline lesions. Of the three *BRAF*<sup>V600E</sup> mutation-positive with histologically proven “no cancer” lesions, the FNAC was SFM (V) in one and malignant (VI) in two. On specimen pathology, the nodule with SFM (V) showed extensive sclerosis and calcifications, and thus led to a diagnosis of degenerative nodule (DN) with calcification. The two nodules with malignant (VI) cytology were completely replaced by cystic and regressive changes. Deeper sections of these two specimens revealed micro-focus suspicion of PTC, leading to a diagnosis of WDT-UMP. It has been reported that ultrasound-guided FNA may lead to the complete vanishing of thyroid nodules, rendering final diagnosis upon surgical histopathology difficult or impossible (16,17).

Of the 1,036 surgical specimens that were assessed, 908 were positive for the *BRAF*<sup>V600E</sup> mutation, and 128 were negative. Thus, the RR of *BRAF*<sup>V600E</sup> mutation-positive nodules was 87.6% (908/1,036). The cancer risk in nodules with *BRAF*<sup>V600E</sup> mutation was 99.7% (905/908) according to the histological results.

As shown in *Table 2*, among the 1,036 surgical specimens, PTCs account for a relatively high percentage in each TBSRTC category (80.0% in ND, 23.5% in benign, 38.9% in AUS/FLUS, 12.5% in FN/SFN, 92.0% in SFM, and 96.4% in malignant). *BRAF*<sup>V600E</sup> mutation was identified in 96.7% of PTCs based on FNA-surgery samples. The *BRAF*<sup>V600E</sup> mutation incidence in PTCs according to the TBSRTC was 70.0% for ND, 100% for benign, 85.7% for AUS/FLUS, 60.0% for FN/SFN, 97.1% for SFM, and 97.6% for malignant. The high rate of *BRAF*<sup>V600E</sup> mutation in histologically proven PTCs may be due to the *BRAF*<sup>V600E</sup> mutation serving as an independent marker of aggressiveness of PTC and affecting the frequency of lymph node metastases (18,19). Therefore, clinicians tend to prioritize patients with FNA-*BRAF*<sup>V600E</sup> mutation for surgery.

### Malignancy risk for each ACR TI-RADS category according to histopathological results

The malignant rates of the 1,036 surgical nodules classified



**Figure 1** Detection of *BRAF*<sup>V600E</sup> mutation in 1,905 FNA samples, also analyzed by cytology and compared with histological results. FNA, fine needle aspiration; ND, nondiagnostic; AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN, follicular neoplasm/suspicious for follicular neoplasm; SFM, suspicious for malignancy; PTC, papillary thyroid carcinoma; BL, benign lesion, including benign lesion and borderline tumors; FTC, follicular thyroid carcinoma; HCC, Hürthle cell carcinoma; MTC, medullary thyroid carcinoma; ATC, anaplastic thyroid carcinoma; MC, metastatic carcinoma; NG, nodular goiter; RT, Riedel thyroiditis; HT, Hashimoto thyroiditis; FA, follicular adenoma; SAT, subacute granulomatous thyroiditis; GD, Graves’ disease; AN, adenomatous nodule; WDT-UMP, well-differentiated tumor of uncertain malignant potential; FT-UMP, follicular tumor of uncertain malignant potential; HCA, Hürthle cell adenoma; DN, degenerative nodule; HCT, Hürthle cell tumor; NIFTP, non-invasive follicular thyroid neoplasm with papillary-like nuclear features; HTT, hyalinizing trabecular tumor.

**Table 2** Correlation of TBSRTC categories with *BRAF*<sup>V600E</sup> mutation and histologically proven PTCs

TBSRTC categories	Surgery	PTCs, n (%)	<i>BRAF</i> <sup>V600E</sup> mutation in histologically proven PTCs, n (%)
I (ND)	25	20 (80.0)	14 (70.0)
II (benign)	17	4 (23.5)	4 (100.0)
III (AUS/FLUS)	18	7 (38.9)	6 (85.7)
IV (FN/SFN)	40	5 (12.5)	3 (60.0)
V (SFM)	75	69 (92.0)	67 (97.1)
VI (malignant)	861	830 (96.4)	810 (97.6)
Total	1,036	935 (90.3)	904 (96.7)

The numbers in parentheses are percentages. TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology; PTC, papillary thyroid carcinoma; ND, nondiagnostic; AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN, follicular neoplasm/suspicious for follicular neoplasm; SFM, suspicious for malignancy.

as TI-RADS 2, 3, 4, 5 were 0, 22.2%, 68.8%, and 98.1%, respectively, according to the postoperative histopathology (Table 3).

### Diagnostic performance of $BRAF^{V600E}$ and ACR TI-RADS

The diagnostic performance, including sensitivity, specificity, PPV, NPV, and accuracy (AC), according to  $BRAF^{V600E}$  mutation analysis, ACR TI-RADS, and the combination of both are summarized in Table 4. On applying a higher threshold for positivity (ACR TI-RADS 5), the sensitivity, specificity, PPV, NPV, and AC for malignancy were 97.1%, 68.9%, 98.1%, 58.8%, and 95.5%, respectively. The combination of TI-RADS 5 and  $BRAF^{V600E}$  mutation analysis showed the best AC (97.5%) and significantly increased the specificity (94.8%) compared to the TI-RADS 5 alone (68.9%).

### Variations of RR and ROM after the inclusion of $BRAF^{V600E}$ mutation test and ACR TI-RADS

The distribution of surgical RR and ROM before and after the implementation of the ACR TI-RADS and  $BRAF^{V600E}$  mutation test is displayed in Table 5. A total of 2,643 nodules underwent FNA biopsies from February 1, 2010, to February 1, 2014, before the ACR TI-RADS

and  $BRAF^{V600E}$  mutation analysis were implemented. In comparison,  $BRAF^{V600E}$  mutation analysis and ACR TI-RADS reports were available for 1,905 FNA samples which were obtained between February 2017, and July 2018. Inclusion of the ACR TI-RADS and  $BRAF^{V600E}$  mutation analysis significantly decreased RR and increased ROM for TBSRTC I–III categories ( $P < 0.05$ ) (Figure 2). The most considerable increase in the ROM (41.5% vs. 80%) was observed for the ND (I) category. A slight decrease in the ROM was found for the FN/SFN (IV) category (54.9% vs. 45.0%). No significant difference was found in the SFM (V) and malignant (VI) categories (Table 5 and Figure 2).

## Discussion

The most common FNA diagnosis rendered in our practice was malignant (VI), which was high at 49.1% (1,299/2,643) from February, 2010 to February, 2014, and even higher at 60.4% (1,150/1,905) from February, 2017 to July, 2018. This is an extremely high proportion of malignant FNA diagnoses in comparison with the average of 7.4% found in 11 reports summarized by Sheffield *et al.* (20). We considered three explanations for the relatively high rate of the malignant category. First, there has been a marked increase in thyroid operations in our hospital. Nearly 4,000 thyroid operations were performed in 2019, whereas approximately 1,500 operations were taken per year from 2000 to 2010. Thyroid clinics have been expanded over the past decade. The explosive growth of ultrasound-suspected thyroid nodules and surgeries may force Chinese endocrinologists to concentrate more on patients with a higher ROM, and postpone treatment of biologically more indolent borderline or precursor tumors to active surveillance, which, at the same time, can help reduce the workload of medical staff. This approach also resulted in achieving an extremely high overall ROM at 94.4% (1,036/1,056, from 2017 to 2018), and thus may be an ideal

**Table 3** ACR TI-RADS categories for surgical thyroid nodules

ACR TI-RADS	Malignant (n=978)	Benign (n=58)	ROM (%)
TI-RADS 2	0	9	0
TI-RADS 3	6	21	22.2
TI-RADS 4	22	10	68.8
TI-RADS 5	950	18	98.1

ACR TI-RADS, American College of Radiology Thyroid Imaging Reporting and Data System; ROM, risk of malignancy.

**Table 4** Diagnostic performance of  $BRAF^{V600E}$  mutation, ACR TI-RADS, and combination

Diagnostic modality	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	AC (95% CI)
$BRAF^{V600E}$	81.8 (79.4–84.2)	95.0 (89.5–99.6)	99.6 (99.2–100.0)	24.6 (19.1–30.2)	75.3 (72.8–77.8)
TI-RADS 5	97.1 (96.0–98.1)	68.9 (56.6–81.2)	98.1 (97.2–98.9)	58.8 (46.8–70.8)	95.5 (94.3–96.8)
Combination	97.7 (96.8–98.6)	94.8 (88.9–99.7)	99.6 (99.3–100.0)	71.4 (61.1–81.7)	97.5 (96.6–98.5)

The numbers in parentheses are percentages. TI-RADS 5 was taken as the diagnostic threshold. Combination =  $BRAF^{V600E}$  + TI-RADS 5. ACR TI-RADS, American College of Radiology Thyroid Imaging Reporting and Data System; PPV, positive predictive value; NPV, negative predictive value; AC, accuracy.

**Table 5** Distribution of surgical RR and ROM before and after implementation of the *BRAF*<sup>V600E</sup> mutation and ACR TI-RADS

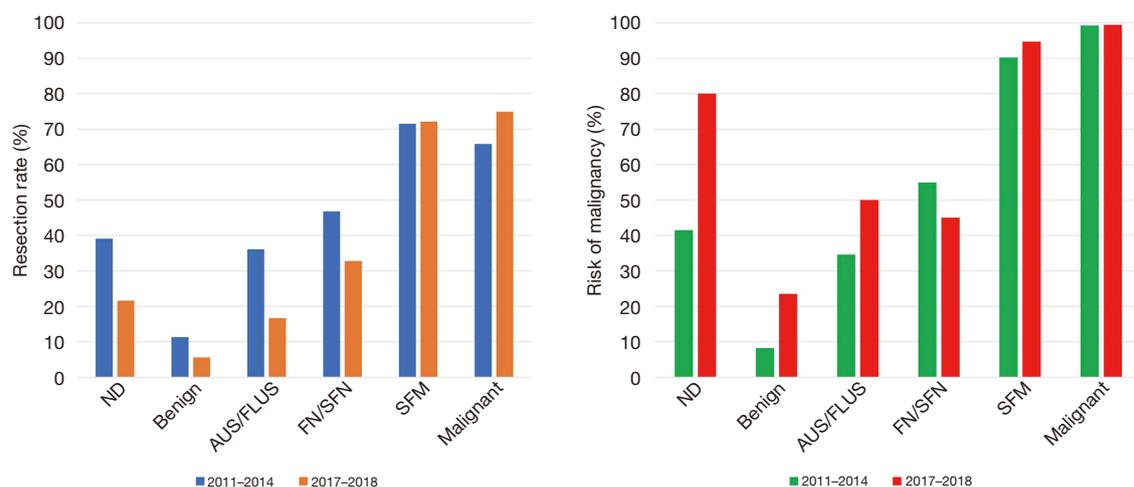
TBSRTC categories	2010–2014, n (%)	2017–2018, n (%)	P value
Diagnoses			
I (ND)	166 (6.3) <sup>a</sup>	116 (6.1) <sup>a</sup>	
RR	65 (39.1) <sup>b</sup>	25 (21.6) <sup>b</sup>	<0.01*
ROM	27 (41.5) <sup>c</sup>	20 (80.0) <sup>c</sup>	<0.01*
II (benign)	753 (28.5) <sup>a</sup>	305 (16.0) <sup>a</sup>	
RR	85 (11.3) <sup>b</sup>	17 (5.6) <sup>b</sup>	0.049*
ROM	7 (8.2) <sup>c</sup>	4 (23.5) <sup>c</sup>	<0.01*
III (AUS/FLUS)	144 (5.4) <sup>a</sup>	108 (5.7) <sup>a</sup>	
RR	52 (36.1) <sup>b</sup>	18 (16.7) <sup>b</sup>	<0.01*
ROM	18 (34.6) <sup>c</sup>	9 (50.0) <sup>c</sup>	<0.01*
IV (FN/SFN)	109 (4.1) <sup>a</sup>	122 (6.4) <sup>a</sup>	
RR	51 (46.8) <sup>b</sup>	40 (32.8) <sup>b</sup>	<0.01*
ROM	28 (54.9) <sup>c</sup>	18 (45.0) <sup>c</sup>	0.012*
V (SFM)	172 (6.5) <sup>a</sup>	104 (5.4) <sup>a</sup>	
RR	123 (71.5) <sup>b</sup>	75 (72.1) <sup>b</sup>	0.874
ROM	111 (90.2) <sup>c</sup>	71 (94.7) <sup>c</sup>	0.098
VI (malignant)	1,299 (49.1) <sup>a</sup>	1,150 (60.4) <sup>a</sup>	
RR	855 (65.8) <sup>b</sup>	861 (74.9) <sup>b</sup>	0.206
ROM	848 (99.2) <sup>c</sup>	856 (99.4) <sup>c</sup>	0.511
Total	2,643	1,905	
RR	1,231 (46.6) <sup>b</sup>	1,056 (54.4) <sup>b</sup>	0.052
ROM	1,039 (84.4) <sup>c</sup>	1,036 (94.4) <sup>c</sup>	0.161

The numbers in parentheses are percentages. <sup>a</sup>, Frequency of thyroid FNA samples in each diagnostic category; <sup>b</sup>, RR among FNA samples in each diagnostic category; <sup>c</sup>, ROM among surgical specimens in each diagnostic category. \*, P<0.05 was considered significant in all tests. ACR TI-RADS, American College of Radiology Thyroid Imaging Reporting and Data System; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology; ND, nondiagnostic; AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN, follicular neoplasm/suspicious for follicular neoplasm; SFM, suspicious for malignancy; ROM, risk of malignancy; RR, resection rate; FNA, fine needle aspiration.

model on how to eliminate unnecessary diagnostic surgery and reduce the overtreatment of indolent thyroid tumors. The high ROM of surgical-treated thyroid nodules is not unusual in Chinese practice (21,22), which is very different from Western countries, where diagnostic surgeries to benign or borderline tumors are common and accepted practice (23,24). The second reason was that all thyroid nodules in the study were classified by ultrasound, and FNAs were performed only when necessary. The third contributing factor might be the fact that our hospital is a thyroid cancer center, which also provides radioactive

iodine (RAI) 131 treatment. Thus, the prevalence of thyroid cancer may be higher at our center compared to that of hospitals in a community hospital setting.

The malignancy rate of each category was found to be much higher than that initially recommended by the TBSRTC, especially in the TBSRTC I, II, and III categories even before the implementation of the ACR TI-RADS and *BRAF*<sup>V600E</sup> mutation analysis (41.5% *vs.* 5–10%, 8.2% *vs.* 0–3%, 34.6% *vs.* 10–30% respectively) (6), indicating that more conservative clinical management or strictly triage patients for surgery was favored. These higher



**Figure 2** The impact of implementing  $BRAF^{V600E}$  mutation and ACR TI-RADS on the relative decrease in the RR and increase in the ROM for TBSRTC categories. ACR TI-RADS, American College of Radiology Thyroid Imaging Reporting and Data System; RR, resection rate; ROM, risk of malignancy; TBSRTC, The Bethesda System for Reporting Thyroid Cytopathology; ND, nondiagnostic; AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN, follicular neoplasm/suspicious for follicular neoplasm; SFM, suspicious for malignancy.

risks of malignancy were also similarly achieved in other Asian practices using the same TBSRTC system (22,25-27). In a recent meta-analysis, Vuong *et al.* analyzed a total of 38 studies with 145,066 fine-needle aspirations and found that Asian series had a significantly higher ROM in TBSRTC I, II, and III categories when compared with Western practice (26.5% *vs.* 13.2%, 13.8% *vs.* 4.1%, 45.9% *vs.* 21.5%) (28). The high ROM in benign or indeterminate categories indicates that decisions to carry out thyroidectomy are thorough in Asian practice (25).

The main aim of thyroid FNA is to assist clinicians in their decisions on the patient's management (29). Benign (II), SFM (V), and malignant (VI) categories have "sufficient for decision" risks of malignancy in our practice (8.2%, 90.2%, and 99.2%, respectively). Subsequently, cytopathological diagnoses in these three categories should give clinicians clear treatment suggestions. The problematic categories of ND (I), AUS/FLUS (III), and FN/SFN (IV) indicate risks of malignancy that are in the indeterminate range of 34.6% to 54.9%, and therefore represent a dilemma in treatment decision-making.

The ACR TI-RADS categories, which include, benign (TI-RADS 1, TI-RADS 2), mildly suspicious (TI-RADS 3), moderately SFM (TI-RADS 4), or highly SFM (TI-RADS 5) (3), can accurately and efficiently predict malignancy risks according to the number of suspicious

ultrasound features (3). The  $BRAF^{V600E}$  mutation is highly specific to PTC, and false-positive tests are rarely reported, making it a powerful adjunct for PTC (30).

Therefore, we have adopted the ACR TI-RADS classification and  $BRAF^{V600E}$  mutation analysis, together with FNAC, as a routine procedure, intending further to assess the risk of the patients with TBSRTC I, III, and IV reports. In our study, the ROM is inclined to increase with advancing TI-RADS category (Table 3). High-risk ultrasound features (TI-RADS 5) did show a good performance in predicting malignancy (98.1%). The combination of TI-RADS 5 and  $BRAF^{V600E}$  mutation reached the best diagnostic efficiency (sensitivity 97.7%, specificity 94.8%, PPV 99.6%). It is apparent that after the implementation of ACR TI-RADS and the  $BRAF^{V600E}$  mutation analysis, the RRs of TBSRTC I and III categories showed significant decreases (39.1% *vs.* 21.6% and 36.1% *vs.* 16.7%, respectively). In contrast, the risks of malignancy of TBSRTC I and III categories indicated substantial increases (41.5% *vs.* 80.0% and 34.6% *vs.* 50.0%, respectively). The ROM of thyroid nodules with the ND (I) category showed the most significant increase from 41.5% to 80.0% after the inclusion of the ACR TI-RADS and  $BRAF^{V600E}$  mutation analysis, indicating such a combination of molecular test with ultrasound features could exhibit high diagnostic value in predicting malignancy and help prevent unnecessary diagnostic surgeries.

Repeated ultrasound-guided FNA, or follow-up ultrasound was recommended for the patients with ND (I) and AUS/FLUS (III) reports (6). Some authors recommended routine repeated FNA or selective FNA to avoid false-negative results (31,32). However, this practice may result in unnecessary FNA and have low cost-effectiveness (33). There are very few repeated FNAs in our practice. Nodules with *BRAF*<sup>V600E</sup> mutation-positive results will be directly recommended for surgery regardless of the cytological reports. For indeterminate nodules with *BRAF*<sup>V600E</sup> mutation-negative results, after excluding clinical high-risk factors, repeated FNA would only be considered for those who have demonstrated three or more suspicious ultrasound features (33,34).

Some limitations to our study should be addressed. Our study was a single thyroid cancer center study, and most of the thyroid nodules in our series had a TBSRTC-VI FNA diagnosis. PTC accounts for a majority of malignant diagnoses (95.6%, 935/978, from 2017 to 2018). As a result, some selection bias was unavoidable. Furthermore, *BRAF*<sup>V600E</sup> mutation has an especially exclusionary role for PTC, whereas the absence of *BRAF*<sup>V600E</sup> mutation does not rule out the presence of PTC (35). Classic PTC commonly exhibits high-suspicion ultrasound patterns (TI-RADS 5). However, follicular carcinoma and follicular variant PTC are present in relatively large proportions among malignant tumors with low-suspicion ultrasound features (3,36,37). The high specificity of TI-RADS 5 and *BRAF*<sup>V600E</sup> mutation for PTC, to some extent, explained why the implementation of ACR TI-RADS and *BRAF*<sup>V600E</sup> mutation did not increase the ROM for the FN/SFN (IV) category since PTCs account for a small percentage of this category (12.5%). In contrast, PTCs account for a relatively high proportion of the ND (80%) and AUS/FLUS categories (38.9%).

The above factors provide ACR TI-RADS and *BRAF*<sup>V600E</sup> mutation analysis with the powerful ability to efficiently identify PTC in each TBSRTC category but might have contributed to an overestimation of our findings. Nevertheless, collecting accurate data from local institutional cytological/histopathological surveys of risk for malignancy in each cytological subcategory remains a worthwhile endeavor, as there is wide inter-institutional variation in the ROM across the various categories (5,28). Moreover, an individualized risk prediction model to accurately assess the cancer risk of patients with cytologically indeterminate thyroid nodules is helpful for clinicians to more reliably select patients for surgery (38). A final limitation of our study is that 10 certified radiologists

performed the ultrasound examination, and thus intra- and interobserver variability in interpreting the ACR TI-RADS reports might have been present (39).

## Conclusions

ACR TI-RADS, *BRAF*<sup>V600E</sup> mutation, and cytological diagnoses, can assist in improving the prediction of thyroid nodule malignancy, especially in the ND (I) and AUS/FLUS (III) categories. Clinicians can apply different management strategies in their use of TBSRTC, and formulating a more individualized treatment schedule may help in making informed decisions on indeterminate nodules and improving diagnostic performance.

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