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The Role of BRAF V600E in Reducing AUS/FLUS Diagnosis in Thyroid Fine Needle Aspiration

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Abstract

BRAF V600E mutations are common in papillary thyroid carcinoma (PTC) and some de-differentiated thyroid cancers. In this study, we summarize AUS/FLUS diagnosed cases from thyroid fine needle aspirations in our center from 2015 to 2017 to explore the impact of BRAF V600E detection on the cytopathological diagnosis of PTC. BRAF V600E detection could significantly reduce the AUS/FLUS diagnosis rates from 11.59 to 8.42% when all BRAF V600E-mutated AUS/FLUS cases were diagnosed as conforming to PTC (20.01 to 19.13% in 2016 and 10.92 to 7.93% in 2017, respectively). The AUS/M rates decreased from 0.67 to 0.64 in 2016 and from 0.33 to 0.23 in 2017. We further discuss a case with a single BRAF V600E cytological mutant lacking a postoperative PTC diagnosis and discuss the limitations of BRAF V600E detection using puncture elution fluid. Our findings support the notion that BRAF V600E detection can effectively reduce the diagnostic rates of AUS/FLUS and help clinicians decide both treatment strategies and patient prognosis.

Keywords BRAF V600E · Thyroid fine needle aspiration · Papillary thyroid carcinoma · AUS/FLUS

Background and Purpose

Fine needle aspiration cytology (FNAC) is a fast, safe, and minimally invasive method to differentiate malignant thyroid nodules from those that are benign. FNAC was first introduced in Scandinavia in the 1950s and gained worldwide popularity in the 1980s [1]. As the accuracy rates of FNAC are as high as 97%, it is regarded as the gold standard first-line diagnostic test for the evaluation of thyroid nodules.

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) was proposed in 2007 and includes six diagnostic categories, each with a suggested risk of malignancy and with recommendations for patient management [2]. The TBSRTC system standardizes the reporting of FNAC, thereby facilitating the communication between pathologists and treating physicians, improving the quality of FNAC reporting. The TBSRTC also reduces ambiguous diagnoses and decreases surgery rates for benign lesions, culminating in its widespread use, international acceptance, and endorsement by the American Thyroid Association (ATA) as part of the revised 2015 ATA guidelines. Despite the benefits of FNAC and the Bethesda systems, limitations still exist, the most significant of which includes cases that fall into the "atypical" category and are termed "atypia of undetermined significance (AUS)" or "follicular lesions of undetermined significance (FLUS)." The malignancy rates for these categories vary amongst institutions, ranging from 15.7 to 81% [3]. The reasons for these discrepancies include the puncture technique limiting the number of smears, failure to perform a clear diagnosis due to inadequate atypical presentation of the lesion, and limitations in the pathologist's diagnostic level. Heterogeneity also exists between cytology subgroups with significantly different risks of malignancy. In the second edition of the TBSRTC released in 2017, the "atypical" category was reclassified into cytological atypia, architectural atypia, cytological and architectural atypia, Hürthle cell-related AUS/FLUS,

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atypia NOS, and atypical lymphocytes (lymphoma should also be considered) [4].

In the 2017 edition of the TBSRTC, molecular tests including BRAF gene detections were recommended as an auxiliary means for cytological and pathological diagnosis [4]. BRAF V600E mutation is a specific gene abnormality observed in papillary thyroid carcinoma (PTC) and some de-differentiated thyroid cancers. To date, BRAF V600E mutation in benign thyroid tumors or nodules has not been reported [5].

From September 2016, our center performed BRAF V600E gene detections in thyroid fine needle aspiration specimens. Herein, we summarize the AUS/FLUS diagnosed cases of thyroid fine needle aspiration in our center from 2015 to 2017 to explore whether BRAF detection in the puncture specimens improves the effectiveness of PTC diagnosis. The application and future perspectives of routine BRAF V600E analysis are discussed.

Methods

Included Cases

A total of 3988 cases of thyroid fine needle aspirations in the Department of Pathology of Chongqing Medical University from December 2015 to 2017 were analyzed. The patients ranged from 9 to 88 years (average 46.71). The ratio of female to males was 3:7.

BRAF V600E Detected in AUS/FLUS Diagnostic Patients

AUS/FLUS diagnosed patients with the nuclear characteristics of PTC were suggested for BRAF V600E gene analysis. RT-PCR was used to detect BRAF V600E mutation in cells scraped from traditional smear/liquid-based cell smears or cell wax blocks. The mutation rates amongst all cases tested for BRAF V600E were calculated.

Comparison of BRAF V600E Mutational Status with the Pathological Diagnosis After Operation

A series of patients underwent surgery and received an accurate histological diagnosis after surgery in both BRAF V600E mutant and BRAF V600E wild-type patients. We further analyzed the postoperative diagnostic categories of the two groups and analyzed the influence of BRAF V600E mutational detection in the puncture specimens of a selection of surgical patients.

Influence of BRAF V600E Detection on AUS/FLUS Diagnosis Rates

BRAF V600E detection permitted a more detailed analysis of the puncture smear specimens. For example, the identification of BRAF V600E mutation prompted us to make a diagnosis of PTC in a patient previously diagnosed with AUS/FLUS lacking cellular morphological support. However, BRAF V600E wild-type results can lead to two diagnoses: (1) PTC due to cytological atypia; or (2) the maintenance of AUS/FLUS diagnosis. According to the above criteria, cases that can be definitely diagnosed as PTC due to BRAF V600E mutation are no longer included in the diagnosis range of AUS/FLUS. We therefore calculated whether BRAF V600E tests reduced the diagnosis rates of AUS/FLUS.

Statistical Analysis

Statistical analysis was performed using a Student's *t* test with SPSS 19.0 statistical software. *P* values < 0.05 were considered statistically significant differences.

Results

BRAF V600E Detection in Cytological Specimens Affects Disease Distribution Following Surgery

The thyroid fine needle puncture case numbers in our center were 130, 1349, and 2509 in 2015, 2016, and 2017 respectively. A total of 574 cases of AUS/FLUS were diagnosed over the 3 years, including 30, 270, and 274 in 2015, 2016, and 2017, respectively (Table 1). Since September 2016, 343 cases of BRAF V600E genetic tests were recommended and 143 tests were performed. Amongst them, 94 cases were mutated and 49 cases were wild type. The mutation rates were 65.73% (Table 2). Finally, 70 mutant cases and 16 wild-type cases were accepted for operation. In 70 of the operated

Table 1 Effects of BRAF V600E mutation detection on AUS/FLUS and AUS/M diagnosis rates in 2015, 2016, and 2017 (BRAF V600E detections were performed in September 2016) (*P<0.05)

Year	2015	2016	2017
The number of cases	130	1349	2509
AUS/FLUS number/rate (before BRAF V600E detection)	30 (23.08%)	270 (20.01%)	274 (10.92%)
AUS/FLUS number/rate (after BRAF V600E detection)	–	258 (19.13%)	192 (7.93%*)
AUS/M rate (before BRAF V600E detection)	1.04	0.67	0.33
AUS/M rate (after BRAF V600E detection)	–	0.64	0.23*

Table 2 Postoperative pathological diagnosis of BRAF V600E detected in AUS/FLUS patients (plus composition ratios)

	BRAF V600E mutated	BRAF V600E wild type
Number	94 (65.73%)	49 (34.27%)
Number (had surgery)	70	16
PTC	69 (98.57%)	14 (87.50%)
Hashimoto's thyroiditis	1 (1.43%)	0 (0%)
Adenoma	0 (0%)	1 (6.25%)
Subacute thyroiditis	0 (0%)	1 (6.25%)

mutant cases, 69 were diagnosed as PTC, and only one case was diagnosed as Hashimoto's thyroiditis with follicular epithelial cell dysplasia. A total of 14 cases were diagnosed as PTC in 16 operated wild-type cases, the other two being adenoma and Subacute thyroiditis (Table 2). The BRAF V600E mutational rates in AUS/FLUS PTC patients confirmed by surgery were 83.13% (Table 3).

BRAF V600E assessments further influenced the clinical treatment of patients. We present two cases: the first is a BRAF V600E mutant case who received surgery and was diagnosed with PTC; the second is a wild-type case in puncture specimens who received an AUS/FLUS diagnosis and was suggested for close follow-up. The patient selected follow-up as opposed to surgery that revealed no increases in nodule size (Fig. 1).

BRAF V600E Detection Can Reduce the Diagnosis Rates of AUS/FLUS

The AUS/FLUS diagnosis rates were 23.08% in 2015, 20.01% in 2016, and 10.92% in 2017. The diagnostic rates of AUS/FLUS significantly decreased after the application of BRAF V600E gene detection, with rates of 19.13% in 2016 and 7.93% in 2017 ($P < 0.05$) (Table 1). AUS/M (AUS/malignancy) is an important index to determine the efficiency of thyroid fine needle puncture diagnosis. The ratio of AUS/M was 1.04 in 2015, 0.67 in 2016, and 0.33 in 2017. In cases in which diagnosis by the BRAF V600E test was reclassified, the ratio changes to 0.64 in 2016 and 0.23 in 2017 ($P < 0.05$) (Table 1). Since the detection of BRAF V600E was not initiated until September 2016, we used this time point as a boundary to calculate the final diagnosis rates of AUS/FLUS during the before and after periods. The AUS/FLUS ratios were 22.45% and 11.59% (before BRAF V600E detection)/8.42% (after BRAF V600E detection) ($P<0.05$), respectively (Table 4). The BRAF V600E mutant rates were 27.41% amongst all AUS/FLUS cases (Table 4).

Table 3 BRAF V600E mutation rates in AUS/FLUS patients with PTC confirmed by surgery

	BRAF V600E mutated	BRAF V600E wild type
Number	69	14
Rita	83.13%	16.87%

Discussion

In the 2017 edition of the Bethesda System for Reporting Thyroid Cytopathology, AUS/FLUS diagnosis was divided into cytologic atypia, architectural atypia, cytologic and architectural atypia, Hürthle cell aspirates, atypia (NOS), and atypical lymphoid cells (to rule out lymphoma) [4]. According to this classification, cases were chosen to detect the BRAF V600E gene, and nearly all belonged to cytologic atypia, cytologic, or architectural atypia types.

Prior to the updated version of the TBRSTC in 2017, changes in AUS/FLUS could be diagnosed according to the 2007 version of the TBRSTC. However, in practice, we found that only those AUS/FLUS patients with papillary carcinoma nuclear cell characteristics had a risk of PTC. We therefore included descriptions of the morphological and structural characteristics of thyroid fine needle aspiration diagnostic reports to better classify the AUS/FLUS lesions. We focused on the addition of descriptions of the characteristics of the nucleus, such as "enlarged and irregular, hyaline chromatin, nuclear furrow, and inclusion bodies" [6]. On occasion, the term "PTC cannot be excluded" was added to the diagnosis. As such, not all patients diagnosed with AUS/FLUS were recommended to undergo BRAF V600E testing. The detection of BRAF V600E was only recommended for patients with characterized morphological features of ground-glass nuclei, nuclear sulci, and nuclear inclusion bodies. We counted all cytological pathological reports (whether BRAF V600E mutant or wild type) of the AUS/FLUS cell diagnosis of patients who underwent surgery and found that the proportion of "PTC cannot be excluded" patients were as high as 83.72%. We therefore proposed that, although not the most comprehensive, further identification of the nuclear characteristics of AUS/FLUS cases could help screen those are PTC. The 2017 revision of the TBSRTC emphasizes the importance of papillary nuclear features in AUS/FLUS diagnostic criteria, which is consistent with our previous experience in daily diagnosis.

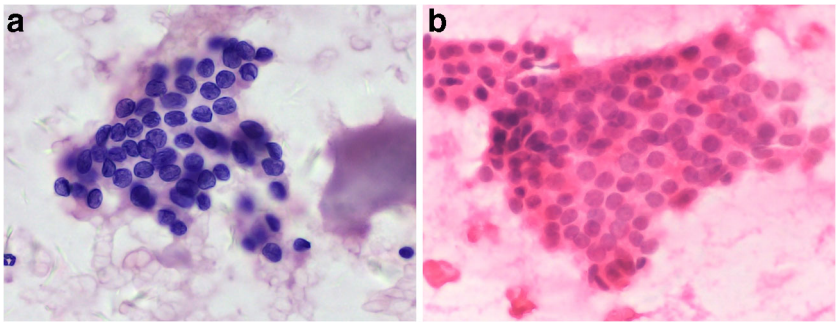

Fig. 1 Two typical cases of AUS/FLUS showing a frosted glass nucleus and groove. **a** Cases of BRAF V600E mutants and subsequent surgery and pathological examination of PTC (Pap staining). **b** Wild-type puncture cells suggestive of an AUS /FLUS diagnosis. The patient chose a follow-up without surgery which revealed no increase in the nodules and no change in the patient's quality of life (H-E staining)

BRAF V600E mutation in AUS/FLUS cases may lead to a diagnosis of "considered PTC" when combined with morphological features, whilst wild type BRAF may still be considered PTC due to excessive abnormal cell morphologies and/or ultrasound changes. As reported, 20–60% of patients with PTC have wild-type BRAF V600E [6, 7]. In multiple studies, mutations in BRAF V600E have been reported as associated with poor clinical outcomes [8–11], whilst some suggest that BRAF V600E may not strongly associate with the prognosis of PTC [12, 13]. In our cases, the diagnosis was made by cell morphology alone and patients diagnosed with PTC underwent surgery. According to our data, the BRAF V600E mutation rates were 83.13%, which were higher than previously reported values [7]. However, we believe that the actual BRAF V600E mutation rates are lower as some BRAF V600E wild-type patients may have been BRAF V600E wild-type PTC patients, but the lesions on the smear were not sufficient for a PTC diagnosis, leading to conservative treatment or repuncture. Our results show that BRAF V600E detection decreased the AUS/FLUS diagnostic rates in 2016 and 2017, particularly in 2017. The AUS/FLUS diagnostic rates also significantly decreased when considering BRAF V600E assessments. The removal of BRAF V600E assessments led to decreased AUS/FLUS diagnostic rates in 2016, and 2017 declined compared with 2015. This decrease was due to the increased understanding of AUS/FLUS lesions by pathologists, improvements in the diagnostic level, and the improvement in puncture and smear preparations. The BRAF V600E mutation rates amongst all AUS/FLUS cases (27.41%) were also higher than those reported in literature, which resulted from the preliminary screening of cases according to cell morphology, in which only those suspected of PTC were assessed for BRAF V600E mutation [14, 15].

We encountered a case in which the cytology was positive but the histology was negative in BRAF V600E mutant/surgery cases. Both smears and tissue sections of this case had a typical Hashimoto's thyroiditis background, accompanied by obvious atypical follicle epithelial cells (Fig. 2). Since to date, there are no reports of BRAF V600E mutation in benign thyroid tumors or nodules. We speculate that the patient might had small and insidious PTC lesions, which remained undiscovered after careful selection.

We have also assessed BRAF V600E mutation using the puncture eluent. We detected BRAF V600E mutation in 180 patients using puncture eluents and cell smear curettage simultaneously. Only two cases of wild-type BRAF were detected in aspiration eluents showing that mutations are frequent in repeated detections of scraped cells from cell smears. We therefore believe that if the puncture eluents are to be used for BRAF V600E detection, quality control issues such as the sufficiency of the sample should first be addressed. A similar scenario has been reported in previous studies [16, 17].

Table 4 Effects of BRAF V600E mutation detection on the AUS/FLUS diagnosis rates from 2015 to 2017 (*P<0.05)

	Without BRAF V600E detection	With BRAF V600E detection
The total number of cases	1029	2959
AUS/FLUS number/rate (before BRAF V600E detection)	231 (22.45%)	343 (11.59%)
AUS/FLUS number/rate (after BRAF V600E detection)	–	249 (8.42%*)
BRAF V600E mutated rate in AUS/FLUS cases	–	27.41%

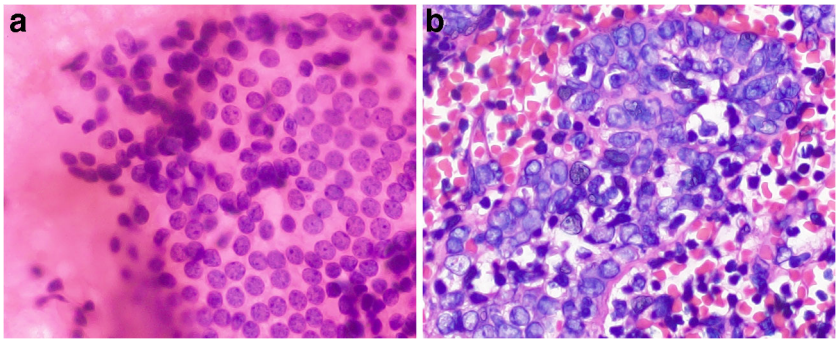

Fig. 2 A case with cytology positive morphological features and mutated BRAF V600E that was histology negative. **a** Smears show obvious atypical follicle epithelial cells with enlarged and irregular nuclei, hyaline chromatin, and nuclear furrows. BRAF V600E is mutated in this sample. **b** Tissue sections showing a typical Hashimoto's thyroiditis background, accompanied by obvious atypical follicle epithelial, scattered into small groups (not enough to diagnose as PTC)

Our findings suggest that BRAF V600E gene detections can effectively reduce the diagnostic rates of AUS/FLUS and improve the positive detection rates of fine needle aspiration. Furthermore, both reasonable application and interpretation of BRAF V600E data can help clinicians decide the optimal treatment strategy and understand patient assessments.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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