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# The Diagnostic Value of *BRAF V600E* Gene Detection Combined with DNA Ploidy Analysis in Thyroid Cancer

Boshu Cui[1], Zaifeng Wu[2], Bing Yu[1], Yuting Li[1], Hongyu Liu[1,*]

[1]Department of Pathology, The First Hospital of Qiqihar, Affiliated Qiqihar Hospital, Southern Medical University, 161000 Qiqihar, Heilongjiang, China
[2]Second Department of Osteology, The First Hospital of Qiqihar, Affiliated Qiqihar Hospital, Southern Medical University, 161000 Qiqihar, Heilongjiang, China
*Correspondence: 15174505123@163.com (Hongyu Liu)
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**Background: The thyroid cancer incidence has been experimenting an accelerated growth all over the world. The serine/threonine-protein kinase (*BRAF*) *V600E* gene detection or the DNA ploidy analysis has been employed in the identification of thyroid cancer type. This study aimed to evaluate the diagnostic value of the *BRAF V600E* gene integrated with DNA ploidy analysis in thyroid cancer.**

**Methods: From August 2022 to May 2023, 400 individuals from the thyroid surgery outpatient department of our hospital were enrolled in this study. The participants were divided into low-risk groups (I+II+III group; n = 200) and high-risk groups (IV+V group; n = 200) based on the Thyroid Imaging Reporting and Data System (TI-RADS). A total of the patients were subjected to the DNA ploidy analysis, the *BRAF V600E* gene detection, or the combination of both techniques. We evaluated the diagnostic value of the above techniques and considered the postoperative pathology results as gold standard for cancer diagnosis. The negative predictive value (NPV), accuracy, specificity, sensitivity, and positive predictive value (PPV) of TI-RADS, *BRAF V600E* gene detection, DNA ploidy analysis, and *BRAF V600E* gene detection joined with DNA ploidy analysis were calculated.**

**Results: Among 400 subjects, 238 presented thyroid cancer and 162 had benign lesions, according to the postoperative pathology results. The obtained sensitivity, specificity, accuracy, PPV, and NPV values of TI-RADS were 55.88%, 58.64%, 57.00%, 66.50%, 47.50%, respectively; of *BRAF V600E* gene detection were 81.93%, 69.75%, 77.00%, 79.92%, 72.44%, respectively; of DNA ploidy analysis were 83.19%, 72.84%, 79.00%, 81.82%, 74.68%, respectively; of *BRAF V600E* gene combined with DNA ploidy analysis were 90.34%, 76.54%, 84.75%, 84.98%, 84.35%, respectively. Compared with TI-RADS, the sensitivity, specificity, accuracy, PPV, and NPV values of DNA ploidy analysis, *BRAF V600E* gene detection, and the conjunction of these last two methods were increased ($p < 0.05$). The combination of DNA ploidy analysis and *BRAF V600E* gene detection had the highest values among them all.**

**Conclusions: *BRAF V600E* gene detection in conjunction with DNA ploidy analysis showed a better diagnostic value than both methods separately or TI-RADS.**

**Keywords:** thyroid cancer; DNA ploidy analysis; *BRAF V600E* gene detection; TI-RADS

## Introduction

As a common endocrine system malignancy, the incidence of thyroid cancer was rapidly elevated in the past 30 years all over the world, and 1.1% increase of mortality happened per year [1–3]. According to the pathological type, thyroid cancer is classified into follicular, undifferentiated, papillary and medullary; in which the primary type malignancy accounts for about 90% of all thyroid cancers [4,5]. It has been pointed out that the diagnosis and therapy for thyroid cancer might be excessive, and only a small amount of patients suffered from extraglandular invasion, lymph node or distant metastasis [6,7]. The prognosis of distant metastasis individuals is poor and its 5-year survival rate is only 57.00% [8]. Thus, the early effective diagnosis and treatment are necessary for the good prognosis of thyroid malignancies.

Currently, the conventional ultrasonography of the thyroid nodules is a widely utilized diagnosis tool characterized by affordable, non-invasive, and reproducible [9]. Thyroid Imaging Reporting and Data System (TI-RADS) was proposed to standardize the thyroid nodule classification according to multiple ultrasonic characteristics such as margins, echogenicity, composition, location, shape, calcifications, size and vascularity. There are multiple guidelines involved in the interpretation of the images pertaining to thyroid ultrasounds [10,11]. Although the features of malignant and benign nodules are different on ultrasound, the images may be misunderstood due to the lack of experience of the examiner or the overlapped features. For example, it was difficult to distinguish the atypical thyroid nodules and the TI-RADS category 4 nodules, which can be difficult to interpret. This poses a challenge to accurately discriminate

malignant and benign lesions [12,13]. On account of these limitations, it is important to improve the accuracy of detection methods in the diagnosis of thyroid cancer.

According to previous studies, the presence of serine/threonine-protein kinase (*BRAF*) *V600E* gene mutation was observed in 32–90% papillary thyroid cancer, 24% anaplastic thyroid cancer, and 9% poorly differentiated malignancy [14–16]. However, this mutation had never been identified in normal tissue, benign lesions or other subtypes of thyroid cancer [17]. Thus, despite the existence of some controversy, *BRAF V600E* gene mutation seemed to be linked to a more aggressive clinicopathological feature of papillary thyroid cancer [18–20]. A study reported that the diagnostic accuracy of *BRAF V600E* gene mutation combined with enhanced computed tomography (CT) was significantly improved relative to enhanced CT alone [21]. In addition, compared with only TI-RADS, the combination of TI-RADS and *BRAF V600E* gene mutation detection contributed to select the more appropriate management for the patients with atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) thyroid nodule [22].

The content of nuclear DNA increases from mildly dysplastic changes to invasive cancer with a different degree of cellular atypia. Therefore, the early tumorous cells can be determined through the DNA content changes. The emergence of DNA aneuploidy is usually a vital early step during carcinogenesis and constitutes a reliable marker of malignancy [23]. DNA ploidy analysis based on image cytometry results is very convenient, objective and possess a high accuracy in diagnosing several malignant cancer types, including oral and lung cancer [24,25]. DNA aneuploidy was reported to be closely related to thyroid epithelial lesions [26]. Nowadays, artificial intelligence (AI) utilizes counting methods and medical images based on deep learning to automatically diagnose lesions or diseases [27–30]. AI-assisted TI-RADS exhibited good efficacy in the diagnosis of thyroid nodules [31]. AI-assisted DNA ploidy analysis that used AI system with deep learning convolutional neural networks, image analysis algorithms, and feature classifiers is also considered to be very useful to evaluate DNA content changes. In the present study, the performance of AI-assisted DNA ploidy analysis of thyroid nodules screening was explored. Moreover, we also evaluated the diagnostic value of *BRAF V600E* gene detection combined with DNA ploidy analysis in thyroid nodules.

## Methods

### *Subjects*

A total of 400 patients who attended the thyroid surgery clinic of our hospital between August 2022 and May 2023 were included in this study for retrospective analysis. The inclusion criteria were the following: (1) $\geq$18 years old; (2) presence of thyroid nodules on ultrasound; (3) complete TI-RADS reports and imaging data; (4) first surgery and pathology confirmation. The exclusion criteria comprehended: (1) detection of other type of cancer; (2) pregnant women; (3) history of neck surgery or radiation; (4) rejection of DNA ploidy analysis or/and *BRAF V600E* gene detection. The study included 136 males and 264 females with ages from 35–76 years (48.3 $\pm$ 15.6).

### *TI-RADS*

The patient was placed in a supine position to fully expose the neck area. Then, it was scanned using the linear array probe. The features of the lesion located on the gland were recorded. Those included echogenicity, composition, size, shape, margin, and calcification. Two ultrasound doctors who had at least 3 years of experience evaluated the TI-RADS reports of 400 patients without possible interference. Suspicious malignancy features included hypo echogenicity, a taller than wide shape, presence of microcalcifications, irregular margins, and solid or almost solid nodule. With the elevation on the number of these features, the risk of malignancy increased, according to the Kwak TI-RADS grading [8]. TI-RADS I: thyroid reexamination with normal thyroid, no nodules, or total surgical resection; TI-RADS II: well-defined benign nodules; TI-RADS III: non-typical benign nodules (risk $<$5%); TI-RADS IV: suspected malignant nodules (risk 5%–85%), including TI-RADS IVa (risk 5%–10%), TI-RADS IVb (risk 10%–50%), TI-RADS IVc (risk 50%–85%); and TI-RADS V: thyroid cancer (risk 85%–100%). Based on the classification criteria, the 400 participants were divided into the low-risk group (TI-RADS I+II+III group, n = 200) and the high-risk group (TI-RADS IV+V, n = 200).

### *Puncture Samples Collection*

After subcutaneous injection of 2% lidocaine local anesthetic, the ultrasound guided puncture was performed in the target nodule of the patients. Then, the repeated five punctures were carried out in different directions. The puncture samples were obtained and added into the PreservCyt solution (National Armament No. 20160550, Thin Prep, Hologic Incorporated, MA, USA).

### *BRAF V600E Gene Detection*

The obtained samples were preserved using the gene detection kit (ADx-BR02; Amoy Diagnostics Co., Ltd., Xiamen, China), and then DNA extraction was conducted, followed by PCR amplification [32]. Employing a 5′fluorcescent dye or fluorescein (FAM) labeled probe, Real Time Quantitative Polymerase Chain Reaction (RT-qPCR) was performed for the amplification of *BRAF V600E* gene. Two criteria were taken into account to define the presence or absence of a *BRAF V600E* mutation in the PCR. S-shaped fluorescein FAM signal amplification curve and Ct value less than 30 were defined as the positive *BRAF V600E* mutation; and non-S-shaped FAM signal amplification curve of the sample and Ct value $\geq$30 indicated a lack of mutation in the gene.

**Table 1. Diagnostic results of the detection methods.**

| Detection method | TI-RADS | | Total |
|---|---|---|---|
| | I+II+III (n = 200) | IV+V (n = 200) | |
| *BRAF V600E* gene detection | | | |
| Positive, n | 44 | 176 | 220 |
| Negative, n | 156 | 24 | 180 |
| DNA ploidy analysis | | | |
| Positive, n | 42 | 147 | 189 |
| Negative, n | 158 | 53 | 211 |
| *BRAF V600E* gene detection + DNA ploidy analysis | | | |
| Positive, n | 53 | 192 | 245 |
| Negative, n | 147 | 8 | 155 |

TI-RADS, Thyroid Imaging Reporting and Data System; *BRAF V600E*, serine/threonine-protein kinase *V600E*.

### *DNA Ploidy Analysis*

After Feulgen nuclei staining of the sample, the image of each nucleus was scanned and analyzed using an artificial intelligence-assisted automatic cell analyzer (LD DNA-ICM II; Landing, Wuhan, China). According to Guillaud's research, the DNA ploidy result was defined as positive based on two parameters: (1) DNA index (DI) >2.5 was considered as aneuploid, where DI represents the ratio of DNA in thyroid cancer cells/normal thyroid epithelial cells; (2) the number of aneuploid cells was not less than three in the specimen [33].

### *Statistical Analysis*

The data in the present study was analyzed using SPSS software (version 26.0, Chicago, IL, USA). The $\chi^2$ test was applied for the comparison of the enumeration results. The sensitivity, specificity, negative predictive value (NPV), accuracy, and positive predictive value (PPV) of TI-RADS, *BRAF V600E* gene detection, DNA ploidy analysis, and these last two methods combined were calculated. This comparison was based on the diagnosis of malignant and benign nodules from the pathological results. $p < 0.05$ was considered statistically significant.

## Results

### *Diagnostic Results of the Detection Methods*

According to the reports of TI-RADS, 400 subjects were divided into low-risk group (I+II+III group; n = 200) and high-risk group (IV+V group; n = 200). Among the 400 patients, 220 patients (55.00%) were *BRAF V600E*-positive. The results of DNA ploidy analysis showed that 189 patients (47.25%) were DNA ploidy-positive. *BRAF V600E* gene detection joined with DNA ploidy analysis was used to diagnose thyroid cancer. We found that there were 245 positive cancer patients (61.25%) (Table 1).

### *Comparison of the Detection Methods with the Pathological Results*

The pathological reports of 400 patients were obtained after surgery. There were 238 cases of thyroid cancer while 162 turn out to be benign. The value of the thyroid cancer predictors NPV, accuracy, specificity, sensitivity, and PPV of TI-RADS were 47.50% (95/200), 57.00% (228/400), 58.64% (95/162), 55.88% (133/238), and 66.50% (133/200), respectively. The NPV, accuracy, specificity, sensitivity, and PPV of *BRAF V600E* gene detection were 72.44% (113/156), 77.00% (308/400), 69.75% (113/162), 81.93% (195/238), and 79.92% (195/244), respectively. The NPV, accuracy, specificity, sensitivity, and PPV of DNA ploidy analysis were 74.68% (118/158), 79.00% (316/400), 72.84% (118/162), 83.19% (198/238), and 81.82% (198/242), respectively. The NPV, accuracy, specificity, sensitivity, and PPV of *BRAF V600E* gene detection joined with DNA ploidy analysis were 84.35% (124/147), 84.75% (339/400), 76.54% (124/162), 90.34% (215/238), and 84.98% (215/253), respectively. DNA ploidy analysis, *BRAF V600E* gene detection or these last two methods combined, significantly increased the thyroid cancer predictors compared with TI-RADS ($p < 0.05$) (Tables 2,3).

## Discussion

In the last few years, the incidence of thyroid cancer has rapidly increased [34,35]. At present, the main preoperative diagnosis method is ultrasound. The images obtained from it allow the display of the malignancy and benignancy of nodules, and analyzing the tissue in a semiquantitative way hardens the assessment [36,37]. The reliability and accuracy of this technique depends on the expertise of the physician, the image quality and the different features of the thyroid cancer types, leading to some uncertainty in the diagnosis [38]. A previous study indicated that the accuracy in the diagnosis of ultrasound in papillary thyroid cancer was 74–82% [32]. Previously, TI-RADS was widely

**Table 2. Comparison of different detection methods with the pathological results.**

| Detection method | Pathological biopsy results | | Total |
|---|---|---|---|
| | Positive (n = 238) | Negative (n = 162) | |
| TI-RADS | | | |
| IV+V, n | 133 | 67 | 200 |
| I+II+III, n | 105 | 95 | 200 |
| *BRAF V600E* gene detection | | | |
| Positive, n | 195 | 49 | 244 |
| Negative, n | 43 | 113 | 156 |
| DNA ploidy analysis | | | |
| Positive, n | 198 | 44 | 242 |
| Negative, n | 40 | 118 | 158 |
| *BRAF V600E* gene detection + DNA ploidy analysis | | | |
| Positive, n | 215 | 38 | 253 |
| Negative, n | 23 | 124 | 147 |

TI-RADS, Thyroid Imaging Reporting and Data System; *BRAF V600E*, serine/threonine-protein kinase *V600E*.

**Table 3. Diagnostic performance of the detection methods for thyroid cancer.**

| | Sensitivity (%) | Specificity (%) | Accuracy (%) | Positive predictive value (%) | Negative predictive value (%) |
|---|---|---|---|---|---|
| TI-RADS | 55.88 | 58.64 | 57.00 | 66.50 | 47.50 |
| *BRAF V600E* gene detection | 81.93 | 69.75 | 77.00 | 79.92 | 72.44 |
| DNA ploidy analysis | 83.19 | 72.84 | 79.00 | 81.82 | 74.68 |
| *BRAF V600E* gene detection + DNA ploidy analysis | 90.34 | 76.54 | 84.75 | 84.98 | 84.35 |

TI-RADS, Thyroid Imaging Reporting and Data System; *BRAF V600E*, serine/threonine-protein kinase *V600E*.

utilized to objectively detect and classify the thyroid nodules, thereby allowing the corresponding treatment [12,39]. The standard of TI-RADS for thyroid cancer detection consisted on low scores for benign nature and high scores for malignancy [40–42]. In this study, the accuracy of the TI-RADS method obtained was 57.00%. Moreover, the sensitivity and specificity of this method in diagnosing malignant and benign thyroid nodules were 55.88% and 58.64%, respectively. However, it was still difficult to utilize for some types of thyroid nodules due to the limitations of the technique.

*BRAF V600E* gene, with a mutation rate of 53.0%–80.6%, was considered as the most specific biomarker for thyroid cancer. Accumulating evidence has proved that *BRAF V600E* was one of the most commonly carcinogenic markers [43]. Moreover, *BRAF V600E* mutation was also associated with mortality, recurrence and distant metastasis in the therapy treatment for thyroid cancer individuals. Therefore, it is regarded as a prospectively effective target for thyroid cancer [44]. Up to now, the diagnostic value of *BRAF V600E* mutation for the differentiation of the benign and malignant lesions is still controversial among indeterminate thyroid nodules. One study revealed that *BRAF V600E* mutation was able to elevate the prediction of malignant nodules [45]. In contrast, a recent meta-analysis comprehending 32 studies, pointed out the low sensitivity of this mutation in predicting the indeterminate nodules [46]. In our work, the positive rate of detection for this genetic mutation was 55.00% of all thyroid nodules, which was little less than the positive rate based on the pathological results (59.50%). We also compared *BRAF V600E* gene detection and TI-RADS in diagnosing thyroid cancer. Previously, the specificity and sensitivity of *BRAF V600E* gene detection were proved to be higher than TI-RADS in thyroid cancer diagnosis [47–51]. Consistently, we found that the accuracy and sensitivity of *BRAF V600E* gene detection were 77.00% and 81.93%, respectively, significantly higher than TI-RADS.

DNA ploidy analysis enabled the determination of the precancerous conditions or early malignancy through assessing the DNA content. The wide use of DNA ploidy analysis in the screening of different malignancies, such as esophageal cancer, haemato-lymphoid neoplasms and lung cancer, has been reported [52–54]. DNA ploidy was considered as a remarkable independent factor for colorectal cancer prognosis. Individuals with DNA diploid malignancy presented a high survival rate while cases with aneuploid malignancy suffered from a poor outcome [55]. Moreover, measurement of DNA ploidy might be able to predict the imminent malignant transformation prior to colon adenocarcinoma development [56]. The application of DNA ploidy analysis in the prediction of thyroid cancer

has also been reported. DNA ploidy analysis can identify the early thyroid nodular lesions of patients [57]. In the present study, AI-assisted DNA ploidy analysis based on an automatic digital pathological cell analyzer was used to diagnose thyroid cancer. Our findings showed that the positive rate of DNA ploidy analysis was 47.25%. The NPV, accuracy, specificity, sensitivity, and PPV of DNA ploidy analysis in predicting thyroid cancer were 74.68%, 79.0%, 72.84%, 83.19%, and 81.82%, respectively, indicating its feasibility in the diagnosis of thyroid cancer.

Based on the results of the present study, we observed that *BRAF V600E* gene detection joined with DNA ploidy analysis significantly improved the NPV, accuracy, specificity, sensitivity, and PPV of the diagnosis compared with TI-RADS. The combination decreased false positive and negative pathological results of TI-RADS alone. However, the limitation of this study consisted on the sample size, and thus expanding the sample size should be appropriate in order to verify the correct optimal boundary value.

## Conclusions

In summary, the data showed that the diagnostic value of DNA ploidy analysis, *BRAF V600E* gene detection, and both techniques combined was better than TI-RADS. *BRAF V600E* gene detection joined with DNA ploidy analysis showed the best diagnostic value in thyroid cancer.

## Availability of Data and Materials

The dataset analyzed during the current study are available.

## Author Contributions

BSC contributed to the conception of the study and wrote the manuscript. ZFW performed the experiment. BY contributed significantly to analysis and manuscript preparation. YTL performed the data analyses. HYL helped perform the analysis with constructive discussions. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Since this is a retrospective cohort study, having no specific intervention, but only using anonymized medical record data and other institutional clinical information that generated results in an aggregate manner. Ethical certification and informed consent for this study was exempted by the ethics committee immunity of the First Hospital of Qiqihar, Affiliated Qiqihar Hospital, Southern Medical University.

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## Conflict of Interest

The authors declare no conflict of interest.

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