The combination of BRAF$^\text{V600E}$ mutation and Chinese Thyroid Imaging Reporting and Data System is helpful in the management of AUS/FLUS thyroid nodules

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Abstract
Purpose To explore the utility of the BRAF$^\text{V600E}$ mutation in combination with the Chinese Thyroid Imaging Reporting and Data System (C-TIRADS) in the management of atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) thyroid nodule (TN).
Methods 138 AUS/FLUS TNs in 129 patients were included. Each TN underwent preoperative BRAF$^\text{V600E}$ mutation analysis and was classified using the C-TIRADS. Histopathologic diagnosis served as reference standard.
Results 46 benign TNs and 92 malignant TNs were identified. The C-TIRADS 4C and 5 (OR = 10.409, $P = 0.000$), BRAF$^\text{V600E}$ mutation (OR = 36.493, $P = 0.000$) were independent predictors of malignant nodules. There were significant differences in malignancy rate among the different C-TIRADS TNs ($P = 0.000$), and these TNs with higher C-TIRADS were associated with increased malignancy rate ($P$ for trend = 0.000). The rate of the nodule with BRAF$^\text{V600E}$ mutation increased with the increase of C-TIRADS ($P$ for trend = 0.001). For AUS/FLUS TNs without BRAF$^\text{V600E}$ mutation, the malignancy rates of the C-TIRADS 3, 4A, 4B, 4C, and 5 were 0%, 21.4%, 20.8%, 70.8%, and 100%, respectively ($P = 0.000$), and the malignancy rate increased from C-TIRADS 3 to C-TIRADS 5 ($P$ for trend = 0.000). C-TIRADS and BRAF$^\text{V600E}$ mutation had similar diagnostic efficacy ($P > 0.05$), and the sensitivity, negative predictive value, and accuracy of the combination were significantly higher than BRAF$^\text{V600E}$ gene or C-TIRADS alone ($P < 0.05$).
Conclusions C-TIRADS can effectively provide risk stratification for AUS/FLUS nodules. The combination is helpful in selecting appropriate management for AUS/FLUS patients.
Keywords Thyroid nodules · Ultrasound · AUS/FLUS · C-TIRADS · BRAF$^\text{V600E}$ mutation

Introduction
Ultrasound (US)-guided fine-needle aspiration (FNA) cytology is a reliable and effective diagnostic approach in patients with thyroid nodules (TNs) [1]. FNA can determine up to 70% of benign and 50% of malignant TNs without subjecting them to surgical resection [2, 3]. Nonetheless, 10–27% of the FNAs present the “atypical” category [2, 4]: either “atypia of undetermined significance” (AUS) or “follicular lesion of undetermined significance” (FLUS). The malignancy rate was assessed to be 5–15% by the current Bethesda system [2, 5, 6], yet 6–48% of these nodules finally were confirmed to be malignant when underwent surgery [2, 7]. Current guidelines advise performing repeat FNA to guide clinical decision-making in these nodules [1]. However, repeat FNA is not an adequate solution due to a significant portion of again AUS/FLUS results and a high false-negative rate [8].

Molecular testing has been proposed in an attempt to improve preoperative risk evaluation of AUS/FLUS nodules [6, 9]. BRAF$^\text{V600E}$ mutation is proven as the most widespread gene mutation in thyroid cancer, especially in papillary thyroid cancer (PTC), with high specificity and positive predictive value (PPV) [9, 10]. When BRAF$^\text{V600E}$ mutation occurs in AUS/FLUS nodules, guidelines recommend surgical procedure.
rather than observed management [1]. Unfortunately, the BRAFV600E gene has limited sensitivity and negative predictive value (NPV) [11, 12], resulting in approximately 33% of cancer undetected. The rate is too high to be beneficial in making decisions between active surveillance and surgery. Meanwhile, the incidence of BRAFV600E mutation in PTC patients was only 35–65% [13], and has not been discovered in follicular thyroid carcinoma (FTC) [14]. Therefore, a challenge remains on the AUS/FLUS nodules tested negative for BRAFV600E mutation in which malignancy fails to be excluded.

US is an effective tool to distinguish malignant from benign AUS/FLUS nodules [15]. Several suspicious US characteristics, such as markedly hypoechoic, infiltrative margin, and microcalcification, can be regarded as predictors of malignancy. Unfortunately, none of these features are sensitive or specific sufficient to be used in isolation. For this reason, several thyroid imaging reporting and data systems (TIRADS) have been formulated to classify TNs and stratify the risk of malignancy. Among them, Zhou et al. established the Chinese-TIRADS (C-TIRADS) as suitable for Chinese clinical practice based on the Chinese TNs US database [16]. C-TIRADS had a sensitivity exceeding 90% and an NPV greater than 95% in a multicentric retrospective study [17]. In addition, compared to other systems, C-TIRADS was simpler and easier to apply [17, 18]. To date, there has been no report to evaluate the value of C-TIRADS for AUS/FLUS nodules.

Our study aimed to assess the ability of C-TIRADS to stratify the malignancy risk for AUS/FLUS nodules and to evaluate the diagnostic performance of C-TIRADS, BRAFV600E mutation, and their combination when applied to AUS/FLUS nodules.

Materials and methods

Patients

The local institutional review board approved this retrospective study, and informed consent was obtained before the procedure. Initially, we reviewed the database of FNA for TNs in our institution from August 2018 to July 2020. The inclusion criteria were as follows: (a) One or more AUS/FLUS cytology results; (b) available US images; (c) underwent BRAFV600E gene testing; (d) surgical resection with a histopathological result matched with the FNA nodule by TN’s location and size. Finally, 138 AUS/FLUS nodules in 129 patients were included (Fig. 1).

Thyroid ultrasound examination and C-TIRADS classification

All US examinations were performed by one of five US experts with more than six years of experience in thyroid US, using Philips IU22, EPIQ5, or GE Logiq E9 devices equipped with a 5–12 MHz linear-array transducer. US images were independently reviewed by two US experts with 10 and 14 years of experience in the thyroid US and blinded to the cytological, BRAFV600E gene testing, and histological findings.

Composition, echogenic foci, echogenicity, margin, and orientation were evaluated in all TNs [16]. The composition was classified into five categories: solid, predominately solid, predominately cystic, cystic, and spongiform. The echogenic foci were classified as microcalcification, comet-tail artifact, punctate echogenic foci of undetermined significance, macrocalcification, peripheral calcification, and no echogenic foci. When a nodule had both microcalcification and other types of echogenic foci, we regarded it as having microcalcification. The TN was considered hyperechoic, isoechoic, or hypoechoic with respect to the thyroid parenchyma or as showing marked hypoechoic when compared with strap muscle. The margin was classified into four categories: circumscribed, irregular margin, ill-defined, or extra-thyroidal extension. Orientation was classified as vertical (taller-than-wide) or horizontal (wider-than-tall) on transverse or longitudinal sections. Solid composition; microcalcification; markedly hypoechoic; irregular margin, ill-defined, or extrathyroidal extension; and vertical orientation were considered the positive US features, while comet-tail artifact showed benign status. Thus, the interpretation of these US features was the same for all US experts.

All TNs were assessed according to C-TIRADS proposed by Zhou [16]. The nodule’s C-TIRADS ranging from C-TIRADS 1 to C-TIRADS 6 was determined by the counting method, being classified as C-TIRADS 1 (no nodule), C-TIRADS 2 (−1 point), C-TIRADS 3 (0 point), C-TIRADS 4A (1 point), C-TIRADS 4B (2 points), C-TIRADS 4C (3–4 points), C-TIRADS 5 (5 points), and C-TIRADS 6 (biopsy proved malignant). When there was no consensus through the independent assessment, a third US expert with 20 years of experience in the thyroid US reviewed the images independently and made a final decision (Table 1).

US-guided FNA and BRAFV600E mutation analysis

FNA indications mainly refer to the 2015 ATA guideline and 2016 AACE/ACE/AME guidelines [1, 19]. In some cases, FNA was performed following the location of nodules, independently from nodule size. In addition, familial thyroid carcinoma, history of radiation exposure during childhood, patient’s personal preference and anxiety level were also taken into account. US-guided FNA was executed with 23- to 25-gauge needles by one of seven US experts with more than six years of experience in FNA.
Each nodule was aspirated at least three passes. FNA samples were smeared onto frosted-end glass slides and immediately fixed in 95% alcohol for hematoxylin and eosin (H&E) staining. Evaluation of FNA samples was executed by a pathologist with more than 10 years of experience in thyroid cytology using Bethesda system criteria [6].

One of the FNA specimens was added to the gene kit (ADx-BR02, Amoy Diagnostics Co. Ltd) followed by DNA extraction and polymerase chain reaction (PCR) amplification (real-time fluorescence quantitative PCR-ARMS was used for detection). The results were analyzed based on the instructions, and mutations in the BRAFV600E gene were obtained.

**Surgical resection and histopathological examination**

Reasons for thyroid surgery were: (a) the presence of another nodule with the FNA diagnosis of suspicious for malignancy or malignant; (b) the results of BRAFV600E testing; (c) poor prognostic factors and the nodule’s location; (d) too large to cause compression symptoms or cosmetic problems; (e) patient’s preference and clinical judgment of the treating physician. Thyroid surgery was performed in our institution and consisted of either lobectomy or total thyroidectomy with central lymph node dissection. If there were lateral lymph nodes metastases, lateral lymph nodes dissection should be
performed. Histopathologic diagnosis was done by board-certified pathologists with more than 10 years of experience in thyroid pathology. They were blinded to the results of US characteristics and BRAFV600E testing. Thyroid cancers were classified according to the 8th edition of the AJCC system [20]. In particular, non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) was considered to be a malignant nodule [21].

Statistical analysis

IBM SPSS statistics (version 19.0, Chicago, IL) were performed for statistical analyses. Normal distribution data were described by mean ± standard deviation (SD), whereas non-normal distribution data were described by a median with 25th and 75th percentiles. The normal distribution data were compared between groups using an independent sample t-test. For non-normal distribution data, differences were analyzed using a Mann–Whitney U test. Qualitative data were presented as frequencies. The risk category, including age, sex, nodule size group, location, coexistence of lymphocytic thyroiditis, C-TIRADS category, AUS vs FLUS, and BRAFV600E status, were compared using the Chi-square test or Fisher’s exact test. Subsequently, the significant factors in univariate analysis were analyzed in logistic regression analysis to assess the odds ratio (OR) for malignancy. To evaluate the linear trend of C-TIRADS among AUS/FLUS, the Cochran–Armitage trend test was performed. A receiver operating characteristic (ROC) curve was constructed, and the area under the ROC curve (AUC) was calculated and compared. $P < 0.05$ was considered statistically significant.

Results

Clinicopathological characteristics of the AUS/FLUS nodules

Of the 138 AUS/FLUS nodules in 129 patients, 92 were pathologically confirmed as malignant, and 46 were benign. Malignant nodules included 80 PTCs, 9 NIFTPs, 2 FTCs, and 1 medullary thyroid carcinoma. The prevalence of NIFTP in our study was 6.5%. Benign nodules included 25 nodular goiters, 5 adenomatous goiters, 4 follicular adenomas, 4 subacute thyroiditis nodules, 4 atypical hyperplasia nodules, 2 lymphocytic thyroiditis nodules, and 2 Hürthle cell adenomas (Table 2).

The patients with malignant nodules comprised 19 men and 73 women (mean age, $45.16 \pm 11.86$ years; age range, $18–74$ years), while benign nodules comprised 6 men and 40 women (mean age, $48.33 \pm 12.64$ years; age range, $19–76$ years). There was a statistical difference in the size of benign and malignant nodules (15.9 $[6.8–20.1]$ mm vs. 10.2 $[7.0–12.0]$ mm, $P = 0.014$). No significant difference in age, sex, nodule size group, location, background of lymphocytic thyroiditis, and AUS or FLUS was found between the two groups (Table 3). Multivariate logistic regression analyzes showed that the C-TIRADS 4C and 5 (OR $= 10.409$, $P = 0.000$), BRAFV600E mutation (OR $= 36.493$, $P = 0.000$) were predictive factors for malignant nodules (Table 3).

Correlation between the BRAFV600E mutation and pathological findings

Among 138 AUS/FLUS nodules, BRAFV600E mutation was observed in 67 nodules, including 64 malignant nodules (64 PTCs) and 3 benign nodules (2 atypical hyperplasia nodules and 1 adenomatous goiter). Negative BRAFV600E mutation was identified in other 71 AUS/FLUS nodules, including 28 malignant nodules and 43 benign nodules (Table 2).

Correlation between the C-TIRADS and pathological findings

The overall distribution of the C-TIRADS for all AUS/FLUS nodules was as follows: C-TIRADS 3 in 6, C-TIRADS 4A in 18, C-TIRADS 4B in 47, C-TIRADS 4C in 65, and C-TIRADS 5 in 2. The malignancy rates in C-TIRADS 3, 4A, 4B, 4C, and 5 were 0%, 33.3%, 57.4%,

Table 1 Chinese Thyroid Imaging Reporting and Data System based on a counting method

| US features                                | Counting value |
|-------------------------------------------|----------------|
| Solid composition                         | +1             |
| Microcalcifications                       | +1             |
| Markedly hypoechoic                       | +1             |
| Irregular margin/ill-defined or extrathyroidal extension | +1            |
| Vertical orientation                      | +1             |
| Negative US features                      | –1             |
| C-TIRADS Category                        | Score          |
| 1, no nodule                              | NA             |
| 2, benign                                 | –1 Point       |
| 3, probably benign                        | 0 Point        |
| 4A, low suspicion                         | 1 Point        |
| 4B, moderate suspicion                    | 2 Points       |
| 4C, high suspicion                        | 3 to 4 Points  |
| 5, highly suggestive of malignancy        | 5 Points       |
| 6, biopsy proved malignant                | NA             |

US ultrasound, C-TIRADS Chinese Thyroid Imaging Reporting and Data System, NA not available
### Table 2 Final pathologic of AUS/FLUS nodules

| Postoperative pathology          | Total $(n = 138)$ | $BRAF^{V600E} (\ast)$ $(n = 67)$ | $BRAF^{V600E} (\neg)$ $(n = 71)$ |
|---------------------------------|------------------|----------------------------------|----------------------------------|
| Benign $(n = 46)$               |                  |                                  |                                  |
| Nodular goiter                  | 25               | 0                                | 25                               |
| Adenomatous goiter              | 5                | 1                                | 4                                |
| Follicular adenoma              | 4                | 0                                | 4                                |
| Subacute thyroiditis            | 4                | 0                                | 4                                |
| Atypical hyperplasia            | 4                | 2                                | 2                                |
| Lymphocytic thyroiditis         | 2                | 0                                | 2                                |
| Hürthle cell adenoma            | 2                | 0                                | 2                                |
| Malignant $(n = 92)$            |                  |                                  |                                  |
| PTC                             | 80               | 64                               | 16                               |
| NIFTP                           | 9                | 0                                | 9                                |
| FTC                             | 2                | 0                                | 2                                |
| MTC                             | 1                | 0                                | 1                                |

*US* ultrasound, *C-TIRADS* Chinese Thyroid Imaging Reporting and Data System, *AUS/FLUS* atypia of undetermined significance or follicular lesion of undetermined significance, *PTC* papillary thyroid carcinoma, *NIFTP* non-invasive follicular thyroid neoplasm with papillary-like nuclear features, *FTC* follicular thyroid carcinoma, *MTC* medullary thyroid carcinoma

### Table 3 Relationships between clinicopathological characteristics and AUS/FLUS nodules

| Variable                  | Pathological findings | $p$-value | Multivariate analysis |
|---------------------------|-----------------------|-----------|-----------------------|
|                           | Benign $(n = 46)$     | Malignant $(n = 92)$ | OR (95% CI) | $p$-value |
| Age (years)               |                       |           |                       |           |
| ≤50                       | 26 (56.5%)            | 58 (63.0%)| 0.459                 |           |
| >50                       | 20 (43.5%)            | 34 (37.0%)|           |           |
| Sex                       |                       |           |                       |           |
| Male                      | 6 (13.0%)             | 19 (20.7%)| 0.274                 |           |
| Female                    | 40 (87.0%)            | 73 (79.3%)|           |           |
| Nodule size group (mm)    |                       |           |                       |           |
| ≤10                       | 15 (32.6%)            | 44 (47.8%)| 0.088                 |           |
| >10                       | 31 (67.4%)            | 48 (52.2%)| 1.358 (0.479–3.849)   | 0.564     |
| Location                  |                       |           |                       |           |
| Upper region              | 13 (28.3%)            | 31 (33.7%)| 0.346                 |           |
| Middle region             | 14 (30.4%)            | 32 (34.8%)|           |           |
| Lower region              | 17 (37.0%)            | 21 (22.8%)|           |           |
| Isthmus region            | 2 (4.3%)              | 8 (8.7%)  |           |           |
| Lymphocytic thyroiditis   |                       |           | 0.695                 |           |
| Negative                  | 33 (71.7%)            | 63 (68.5%)|           |           |
| Positive                  | 13 (28.3%)            | 29 (31.5%)|           |           |
| C-TIRADS$^a$              |                       |           | 0.000                 |           |
| Non-suspicious            | 38 (72.6%)            | 33 (35.8%)| Ref                  |           |
| Suspicious                | 8 (17.4%)             | 59 (64.2%)| 10.409 (3.589–30.192)| 0.000     |
| AUS/FLUS                  |                       |           |                       |           |
| AUS                        | 19 (41.3%)            | 49 (53.3%)| 0.185                 |           |
| FLUS                      | 27 (58.7%)            | 43 (46.7%)|           |           |
| $BRAF^{V600E}$             |                       |           |                       |           |
| Positive                  | 3 (6.5%)              | 64 (69.6%)| 0.000                 | 36.493 (9.439–141.092)| 0.000     |
| Negative                  | 43 (93.5%)            | 28 (30.4%)| Ref                  |           |

$^a$The suspicious includes C-TIRADS 4C and 5, non-suspicious includes C-TIRADS 3, 4A and 4B
and their combination with BRAFV600E mutation for AUS/FLUS nodules was as follows: C-TIRADS 3 in 0%, C-TIRADS 4A in 22.2%, C-TIRADS 4B in 48.9%, C-TIRADS 4C in 61.5%, and C-TIRADS 5 in 0%, respectively (P = 0.000), and the rate of the nodule with BRAFV600E mutation increased from C-TIRADS 3 nodules to C-TIRADS 4C nodules (P for trend = 0.001).

For AUS/FLUS nodules without BRAFV600E mutation, C-TIRADS was classified as C-TIRADS 3 in 6, C-TIRADS 4A in 14, C-TIRADS 4B in 24, C-TIRADS 4C in 25, and C-TIRADS 5 in 2, respectively. The malignant rates of C-TIRADS 3, 4A, 4B, 4C, and 5 were 0%, 21.4%, 20.8%, 70.8%, and 100%, respectively (P = 0.000), and the malignancy rate increased from C-TIRADS 3 nodules to C-TIRADS 5 nodules (P for trend = 0.000) (Table 4). The diagnostic performance of BRAFV600E mutation, C-TIRADS, and the combination were significantly better than BRAFV600E gene or C-TIRADS alone (all P < 0.05) (Table 5 and Fig. 2). Among them, a total of 18 AUS/FLUS nodules were misdiagnosed, including 10 false-positive nodules (1 benign nodule showed BRAFV600E mutation and C-TIRADS 4C, 2 showed BRAFV600E mutation, and 7 showed C-TIRADS 4C) and 8 false-negative nodules (3 malignant nodules showed C-TIRADS 4A, and 5 showed C-TIRADS 4B) (Table 6).

### Diagnostic performance of the BRAFV600E mutation, C-TIRADS, and their combination

The sensitivity, specificity, PPV, NPV, accuracy, and AUC of BRAFV600E mutation were 69.6%, 93.5%, 95.5%, 60.6%, 77.5%, and 0.815, respectively. The best cut-off of C-TIRADS was C-TIRADS 4C according to the ROC curve. The sensitivity, specificity, PPV, NPV, accuracy, and AUC of C-TIRADS were 64.1%, 82.6%, 88.1%, 53.5%, 70.3%, and 0.783, respectively, and the diagnosis performance was similar to the BRAFV600E mutation (P > 0.05). The sensitivity, specificity, PPV, NPV, accuracy, and AUC of the combination were 91.3%, 78.3%, 89.4%, 81.8%, 87.0%, and 0.848, respectively. The sensitivity, NPV, and accuracy of the combination were significantly higher than BRAFV600E gene or C-TIRADS alone (all P < 0.05) (Table 5 and Fig. 2). Among them, a total of 18 AUS/FLUS nodules were misdiagnosed, including 10 false-positive nodules (1 benign nodule showed BRAFV600E mutation and C-TIRADS 4C, 2 showed BRAFV600E mutation, and 7 showed C-TIRADS 4C) and 8 false-negative nodules (3 malignant nodules showed C-TIRADS 4A, and 5 showed C-TIRADS 4B) (Table 6).

### Discussion

AUS/FLUS is considered the “gray zone” of cytopathology, including atypia that cannot be readily assigned to a definitive...
cytological category due to architectural and/or nuclear atypia and sample preparation artifact. Previous studies indicated significant differences in the prevalence and malignancy in surgical samples for AUS/FLUS nodules [2, 4–7]. With these heterogeneous findings, the optimal approach to the management of these nodules remains controversial.

In our study, which included a larger surgically confirmed population, the malignancy rate was 66.7%, which is higher than previous reports [5–7], but lower than the other studies [22, 23], and similar to some other studies [24, 25]. This variability was caused by differences in inclusion criteria, modalities of preoperative assessment, and follow-up strategy. The prevalence of NIFPT in our study was in line with that reported in the meta-analysis by Bongiovanni et al. [21]. The size of benign and malignant AUS/FLUS nodules was different (P = 0.014). These results were consistent with Suh et al. [26]. The difference in the size of benign and malignant nodules might be based on the fact that the US had high sensitivity and could detect many malignant nodules smaller than 10 mm. In addition, a proportion of benign nodules performed surgery due to tracheal compression or cosmetic reasons. Using multivariate logistic regression analysis, we found that C-TIRADS 4C and 5 (OR = 10.409, P = 0.000), BRAFV600E mutation (OR = 36.493, P = 0.000) independently predicted the risk of malignancy in AUS/FLUS nodules. This conclusion was consistent with the report of Suh et al. that BRAFV600E mutation was predictive of malignancy in these nodules [26]. However, reports on C-TIRADS predicting the malignancy of AUS/FLUS nodules are scarce.

Currently, BRAFV600E gene has been confirmed as a highly specific marker of thyroid cancer. Compared with a multi-gene panel or next-generation sequencing, BRAFV600E test was already quite established in clinical

![Fig. 2 ROC curve for BRAFV600E gene, C-TIRADS, and their combination diagnosis of AUS/FLUS nodules](image)

| Table 6 Characteristics of patients who showed false-positive or false-negative by BRAFV600E gene combined with C-TIRADS |
|---------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| NO | Age (years) | Sex | US size (mm) | C-TIRADS | BRAFV600E status | Histology |
| 1 | 51 | F | 21 | 4B | Positive | Atypical hyperplasia |
| 2 | 70 | F | 17 | 4A | Positive | Adenomatous goiter |
| 3 | 37 | F | 9 | 4C | Positive | Atypical hyperplasia |
| 4 | 57 | F | 4 | 4C | Negative | Atypical hyperplasia |
| 5 | 32 | F | 6 | 4C | Negative | Atypical hyperplasia |
| 6 | 57 | F | 47 | 4C | Negative | Nodular goiter |
| 7 | 73 | F | 19 | 4C | Negative | Subacute thyroiditis |
| 8 | 41 | F | 19 | 4C | Negative | Adenomatous goiter |
| 9 | 37 | F | 16 | 4C | Negative | Adenomatous goiter |
| 10 | 31 | F | 5 | 4C | Negative | Adenomatous goiter |
| 11 | 38 | F | 6 | 4B | Negative | PTC |
| 12 | 41 | F | 7 | 4B | Negative | PTC |
| 13 | 48 | F | 36 | 4B | Negative | PTC |
| 14 | 55 | F | 6 | 4B | Negative | PTC |
| 15 | 68 | F | 7 | 4A | Negative | PTC |
| 16 | 26 | F | 11 | 4B | Negative | PTC |
| 17 | 22 | F | 33 | 4A | Negative | FTC |
| 18 | 43 | F | 26 | 4A | Negative | FTC |

US Ultrasound, C-TIRADS Chinese Thyroid Imaging Reporting and Data System, PTC papillary thyroid carcinoma, FTC follicular thyroid carcinoma
application and was more cost-effective. In our study, BRAFV600E mutation was identified in 48.6% of AUS/FLUS nodules and 69.6% of malignant AUS/FLUS nodules, which were higher than that described by Xing [10], but approached the described by Kim et al. [27]. The possible reasons for the differences were diverse races, geographic areas, methods of detecting gene mutation, and iodine intake. The specificity and PPV of the BRAFV600E mutation were 93.5% and 95.5%, respectively, which is consistent with the previous report [10]. However, BRAFV600E gene has limited sensitivity and NPV [11]. In our study, BRAFV600E mutation demonstrated a sensitivity and NPV of 69.6% and 60.6%, respectively, which was similar to the reports.

Coincidentally and interestingly, three surgery-proven benign AUS/FLUS nodules harbored BRAFV600E mutation. Two AUS/FLUS nodules were confirmed as atypical hyperplasia, which embraced some cytological characteristics of PTC. We believed that these nodules were identical to the atypical hyperplastic nodule reported by Chung et al. and it might be the precursor of PTC [28]. The other was proven to be an adenomatoid nodule. The high sensitivity of BRAFV600E test might explain the false-positive result [29].

We attempted to stratify the risk of AUS/FLUS nodules based on C-TIRADS guideline [16]. In our study, the malignancy rate of C-TIRADS 3 was 0%, and this value was consistent with the expected malignancy risk by the C-TIRADS guideline. This finding favors follow-up management instead of repeat FNA or surgery for these nodules. However, the number of C-TIRADS 3 nodules was small, and further researches with a large population are required. The malignancy rate of the C-TIRADS 4A and 4B nodules were 33.3% and 57.4%, respectively, and these values were higher than those described in the C-TIRADS guideline. This disparity might be due to selection bias since our study only contained AUS/FLUS nodules, while C-TIRADS guideline suggested a risk stratification for all TNs. However, the malignant rates of C-TIRADS 4A and 4B for AUS/FLUS nodules without BRAFV600E mutation were 21.4% and 20.8%, respectively, which was relatively lower compared with all C-TIRADS 4A and 4B nodules. Thus, C-TIRADS 4A and 4B AUS/FLUS nodules with BRAFV600E mutation can be surgical treatment, while the management of AUS/FLUS nodules without BRAFV600E mutation will depend on the physician’s preference, including follow-up, repeat FNA, and surgery. For C-TIRADS 4C and C-TIRADS 5 nodules, the malignancy rates were very high (70.8–100%) regardless of the occurrence of BRAFV600E mutation, which was consistent with the expected malignancy risk by the C-TIRADS guideline. In addition, there were significant differences in malignancy risk among the different C-TIRADS, and these nodules with higher C-TIRADS were associated with the increased malignancy rate. These findings suggested that C-TIRADS could provide risk stratification for benign and malignant AUS/FLUS nodules, especially in China where genetic test is not yet widely available.

It was important to note the association between C-TIRADS and BRAFV600E mutation. Over two-thirds of C-TIRADS 4C nodules were detected to have BRAFV600E mutation, and BRAFV600E mutation nodules were more likely to be categorized into one of the higher C-TIRADS. In addition, the diagnostic performance of C-TIRADS was similar to BRAFV600E mutation. Therefore, BRAFV600E gene can offer a more cost-effective strategy in AUS/FLUS with C-TIRADS 4A or 4B. Using the combination of BRAFV600E mutation with C-TIRADS 4C in AUS/FLUS nodules, the combination could get an increased sensitivity, NPV, and accuracy in comparison with BRAFV600E test or C-TIRADS alone, which showed that the combination could promote the management of AUS/FLUS patients.

There are several limitations of this study. First, there was a selection bias because we did not include AUS/FLUS nodules that had no pathological findings. It might be a reason for the malignant rate of our study being higher than Bethesda system. However, this aspect is difficult to overcome, due to the fact that the reference criterion must be the postoperative pathology. Second, US features were categorized based on static images rather than actual images, which might influence the evaluation of US features. Third, the sub-classification of the atypia recommended by the 2017 revision was not considered in our study because this did not affect the management of patients. Fourth, our study focused on the BRAFV600E mutation for the diagnosis of AUS/FLUS nodules and did not involve the detection of other genes. Finally, this study was a single-center retrospective study, with ensured consistency of diagnosis outcome.

In conclusion, this study demonstrated that C-TIRADS could effectively provide risk stratification for AUS/FLUS nodules, and the diagnostic performance of C-TIRADS was similar to BRAFV600E mutation. C-TIRADS may be advised as an alternative method for providing information to manage patients with AUS/FLUS nodules, especially in circumstances in which molecular testing is not available. The combination could promote the diagnostic performance of AUS/FLUS nodules, and it is helpful in selecting appropriate clinical management in AUS/FLUS patients.

Data availability

All data generated or analyzed during this study are included in this article.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were
performed by Q.L., L.Y., J.L., L.X., M.Z. The first draft of the manuscript was written by Q.L. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare no competing interests.

**Ethical approval** This study was approved by the board of medical ethics of Sir Run Run Shaw Hospital, Zhejiang University, School of Medicine.

**Consent to participate** Written informed consents were obtained from all participants included in the study.

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