

Peer Review File

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Reviewer #1

Comment 1: It is a very interesting study. I would suggest authors modify their writing so that the manuscript becomes more reader-friendly. See attached.

Reply 1: The sentence/paragraph marked by the reviewer has been rewritten and additional proofreading has been done. I am quite willing to fix if there are any questions about the writing. Thank you for the comments.

Reviewer #2

This manuscript by Zhu et al. is a well-written validation study of the diagnostic performance of ACR TI-RADS and BRAFV600E mutation analysis based on the corresponding FNAC and surgical histopathology results in a Chinese population. This study highlighted an excellent performance of the BRAF single gene test particularly in inadequate and AUS cytological categories, and achieved a very high overall ROM in resected thyroid nodules.

Major comments:

Comment 1: Please state in the introduction section that this is a validation study of the diagnostic performance of ACR TI-RADS, FNA cytology, and BRAFV600E molecular test in a Chinese population.

Reply 1: I have added the description of "This is a validation study of the diagnostic performance of ACR TI-RADS, FNA cytology, and BRAF^{V600E} molecular test in a Chinese population" in the introduction section.

Page 5, in the introduction section, "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 resection rate and risk of malignancy for TBSRTC before and after the implementation of ACR TI-RADS and BRAF^{V600E} mutation analysis at our institution. **This is a validation study of the diagnostic performance of ACR TI-RADS, FNA cytology, and BRAF^{V600E} molecular test analysis in a Chinese population.**"

Comment 2: Please show background data of this practice, such as 1) the total number of patients with thyroid nodules who underwent US examination, 2) total number of FNA and its indication (please cite any clinical guidelines if any), 3) a total number of BRAF tests using FNA samples, 4) the number of surgery and how to triage patient for surgery (please cite any clinical guidelines if any), and 5) exclusion criteria from this study (how many cases were excluded and reasons). You may exclude patients who were referred to the Jiangsu Institute for surgical treatments and whose clinical tests were carried out in other hospitals or not available for study.

Reply 2: I am sorry I did not describe the study population clearly in the Methods

section. A new table (Table 1) has been added for the clinical data of the study population. The information of the background data includes 1) the total number of patients with thyroid nodules who underwent US examination, 2) total number of FNA and its indication, 3) a total number of BRAF tests using FNA samples, 4) how to triage patient for surgery and 5) exclusion criteria from this study was explained in the Methods section.

Page 6-7, in the Methods section, "This was a **retrospective** study. The protocol was reviewed and approved by the Institutional Review Board of Jiangsu Institute of Nuclear Medicine (IRB No. YL201601). **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, 2,643 consecutive thyroid nodules from 2,399 patients (688 men and 1711 women) underwent preoperative ultrasound-guided or palpation guided FNA biopsies. All patients have thyroid ultrasound records available. During this period, all thyroid nodules did not undergo ACR TIRADS stratification and the BRAF^{V600E} mutation test. The average age was 44.3 ± 12.5 years old, and the mean diameter was 1.4 ± 0.9 cm (*Table 1*). The cytological reports were adjusted with the 2009 Bethesda system and classified into six categories.

The institution has adopted TI-RADS stratification and BRAF^{V600E} mutation analysis as a routine procedure since January 2016. Between February 1, 2017, and July 31, 2018, 2167 consecutive thyroid nodules of 2,011 patients underwent preoperative ultrasound-guided FNA biopsies. During this period, nodules that qualified the following criteria were included in this survey: 1) thyroid cytology based on the six-tiered 2009 Bethesda system; 2) availability of BRAF^{V600E} mutation analysis; 3) an ultrasound examination of the nodules which were classified by the five-tiered ACR TI-RADS. Finally, a total of 1,905 thyroid nodules of 1,837 patients (501 men and 1336 women) were enrolled. 262 out of 2167 nodules were excluded from the survey because patients refused to undergo molecular tests, or the ultrasound contains only a descriptive report without ACR TI-RADS grading. The average age was 49.5 ± 12.8 years old, and the mean diameter was 1.0 ± 0.7 cm. The difference in average age between the period of 2010-2014 and 2017-2018 was significant, as well as nodule diameter ($P < 0.01$) (*Table 1*)."

Page 8-9 in the Methods section, "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 ≥ 2.5 cm and TI-RADS 4 nodules measuring ≥ 1.5 cm were recommended for FNA biopsy (11).** Other factors indicating for FNA included **patients who had been exposed to previous radiation to the neck, those with a family history of thyroid cancer, and the nodule grows during the surveillance (8).** **Thyroid surgery was recommended to all patients with FNA-BRAF^{V600E} mutation positive cases regardless of the cytological reports. For BRAF^{V600E} mutation negative cases, patients with suspicious for malignancy (V) or malignant (VI) were recommended for surgeries. Repeated ultrasound-guided FNA or follow-up ultrasound was**

recommended for the patients with Nondiagnostic (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 with a TI-RADS 2 or TI-RADS 3 scoring may be suggested for active surveillance. Patients with benign (II) reports were commonly recommended for ultrasound follow up except for hyperfunctioning goiter, pressure symptoms, or influencing the appearance."

Reference

8. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 2016;26:1-133.

11. Kwak JY, Han KH, Yoon JH, et al. Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. *Radiology* 2011;260:892-9.

Comment 3: As an explanation for an extremely high proportion of the malignant FNA category, the author mentioned an excessive hospital workload and a workforce shortage in the discussion section. However, it was a negative reason which may not be useful for other readers, particularly for those in Western practice. Please discuss it in more positive ways, such as how Chinese endocrinologists accurately select high-risk nodules for FNA and triage high-risk patients for surgery, which was helpful to reduce work overload to the medical staff at the same time. This approach also resulted in achieving extremely high overall ROM, which created an ideal performance on how to eliminate unnecessary diagnostic surgery and reduce the overtreatment of indolent thyroid tumors. It was very different from those in Western series, where diagnostic surgeries to benign or borderline tumors were common practice and were accepted (Ferris RL, Nikiforov Y, Terris D, et al.: AHNS Series: Do you know your guidelines? AHNS endocrine section consensus statement: State-of-the-art thyroid surgical recommendations in the era of noninvasive follicular thyroid neoplasm with papillary-like nuclear features. *Head Neck* 40:1881-1888, 2018. Haugen BR, Sawka AM, Alexander EK, et al.: American Thyroid Association guidelines on the management of thyroid nodules and differentiated thyroid cancer task force review and recommendation on the proposed renaming of encapsulated follicular variant papillary thyroid carcinoma without invasion to noninvasive follicular thyroid neoplasm with papillary-like nuclear features. *Thyroid*. 2017; 27:481-483.)

Reply 3: I have rewritten the explanation for an extremely high proportion of the malignant FNA category according to your comments.

Page 16-17, in the discussion section, " **We considered three explanations for the relatively high rate of 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 the Chinese endocrinologists to concentrate more on patients with a higher risk of malignancy, and postpone treatment of biologically more indolent borderline or precursor tumors, as active surveillance, which was helpful to reduce work overload to the medical staff at**

the same time. This approach also resulted in achieving an extremely high overall risk of malignancy at 94.4% (1036/1056, from 2017 to 2018), which created an ideal performance on how to eliminate unnecessary diagnostic surgery and reduce the overtreatment of indolent thyroid tumors. The high risk of malignancy of surgical-treated thyroid nodules was not unusual in Chinese practice (20,21). It was very different from those in Western series, where diagnostic surgeries to benign or borderline tumors were common practice and were accepted (23,24). The second reason was that all thyroid nodules were classified by the ultrasound, and FNAs were performed only when necessary. The third contributing factor might be as 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 those in other hospitals in a community hospital setting. "

Reference

20. Liu Z, Liu D, Ma B, et al. History and Practice of Thyroid Fine-Needle Aspiration in China, Based on Retrospective Study of the Practice in Shandong University Qilu Hospital. *J Pathol Transl Med* 2017;51:528-32.
21. Ke J, Jianyong L, Ying L, et al. The use of The Bethesda System for Reporting Thyroid Cytopathology in a Chinese population: An analysis of 13 351 specimens. *Diagn Cytopathol* 2019;47:876-80.
23. Haugen BR, Sawka AM, Alexander EK, et al. American Thyroid Association Guidelines on the Management of Thyroid Nodules and Differentiated Thyroid Cancer Task Force Review and Recommendation on the Proposed Renaming of Encapsulated Follicular Variant Papillary Thyroid Carcinoma Without Invasion to Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features. *Thyroid* 2017;27:481-3.
24. Ferris RL, Nikiforov Y, Terris D, et al. AHNS Series: Do you know your guidelines? AHNS Endocrine Section Consensus Statement: State-of-the-art thyroid surgical recommendations in the era of noninvasive follicular thyroid neoplasm with papillary-like nuclear features. *Head Neck* 2018;40:1881-8.

Minor comments:

Comment 4: Please describe 3 cases with BRAF false-positive results more in detail to clarify the pitfalls of this approach.

Reply 4: Three cases with BRAF false-positive results were explained in detail in the result section.

Page 12, in the result section, "Of the three BRAF^{V600E} mutation positive with histologically proved "no cancer" lesions, the FNA cytology was SFM (V) in one, Malignant (VI) in two. On specimen pathology, the nodule with SFM (V) showed extensive sclerosis and calcifications, therefore lead to a diagnosis of degenerative nodule 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, lead to a diagnosis of WDT-UMP. It is reported ultrasound-guided FNA may lead to the complete vanishing of thyroid nodules, rendering final diagnosis upon surgical histopathology difficult or impossible (16,17)."

Reference

16. Eze OP, Cai G, Baloch ZW, et al. Vanishing thyroid tumors: a diagnostic dilemma after ultrasonography-guided fine-needle aspiration. *Thyroid* 2013;23:194-200.
17. Bhatia P, Deniwar A, Mohamed HE, et al. Vanishing tumors of thyroid: histological variations after fine needle aspiration. *Gland Surg* 2016;5:270-7.

Comment 5: This reviewer believes that the proportion of BRAFV600E mutation is high in this patient series. Please describe BRAFV600E mutation-positive rate in all PTCs (from Bethesda category I to VI).

Reply 5: A new table (Table 2) has been added to describe BRAF^{V600E} mutation-positive rate in all PTCs (from Bethesda category I to VI).

Page 12-13, in the result section, “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 Nondiagnostic, 23.5% in Benign, 38.9% in AUS/FLUS, 12.5% in FN/SFN, 92.0% in SFM, 96.4% in Malignant). BRAF^{V600E} mutation was identified in 96.7% of PTCs based on FNA-surgery samples. The BRAF^{V600E} mutation incidence in PTCs according to the TBSRTC was 70.0% for Nondiagnostic, 100% for Benign, 85.7% for AUS/FLUS, 60.0% for FN/SFN, 97.1% for SFM, 97.6% for Malignant. The high rate of BRAF^{V600E} mutation in histological proved PTCs may be due to BRAF^{V600E} mutation appears to serve as an independent marker of aggressiveness of PTC and concerning the frequency of lymph node metastases (18,19). Therefore, the clinicians tend to provide a higher priority to patients with FNA-BRAF^{V600E} mutation for surgery. ”

Reference

18. Elisei R, Ugolini C, Viola D, et al. BRAF(V600E) mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. *J Clin Endocrinol Metab* 2008;93:3943-9.

19. Kim SJ, Lee KE, Myong JP, et al. BRAF V600E mutation is associated with tumor aggressiveness in papillary thyroid cancer. *World J Surg* 2012;36:310-7.

Comment 6: Please specify benign lesions in Figure 1, and describe how many borderline tumors there were in each cytological category. In other words, please describe benign diseases (thyroiditis, cyst, hyperplastic nodule, follicular adenoma, etc.) and borderline (precursor) lesions (NIFTP, UMP, and hyalinizing trabecular tumor) separately.

Reply 6: Benign lesions and borderline tumors have been added in Figure 1.

Page 11, in the result section, “As shown in *Figure 1*, The percentages of BRAF^{V600E} mutation rates in 1,905 FNA samples, which were classified into one of the TBSRTC I-VI categories, were 12.9%, 1.3%, 5.6%, 2.6%, 79.8%, and 82.2%, respectively.

1,036 nodules out of 1,905 had final histological diagnoses, and 978 were found to be malignant. The institution has adopted the borderline tumor category introduced by the latest World Health Organization classification of endocrine organs (15) in routine practice, including 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). Of the 1,036 surgical-treated specimens, borderline tumors account for a minimal proportion in each TBSRTC category, none in Nondiagnostic (I) and Benign (II), two (2/18) in AUS/FLUS (III), five (5/40) in FN/SFN (IV), one (1/75) in SFM (V) and two (2/861) in Malignant (VI) (*Figure 1*). ”

Reference

15. Lloyd RV, Osamura RY, Klöppel GK, et al. WHO Classification of Tumours. Pathology and Genetics of Tumours of Endocrine Organs. IARC Press, Lyon, 2017.

Comment 7: Please add comments highlighted with red letters in the following sentences for readers' better understanding in the discussion section. "The shortage of laboratory personnel may force the clinicians to concentrate more on patients with a higher risk of malignancy, and postpone treatment of biologically more indolent borderline or precursor tumors, **as active surveillance**. Another factor might be as our hospital is a thyroid cancer center, which could also provide radioactive iodine (RAI) 131 treatment. The prevalence of thyroid cancer may be higher than **those in other hospitals** in a community hospital **setting**."

Reply 7: The sentences have been modified according to your comments as follows,

Page 16, in the discussion section, "The explosive growth of ultrasound suspected thyroid nodules and surgeries may force the Chinese endocrinologists to concentrate more on patients with a higher risk of malignancy, and postpone treatment of biologically more indolent borderline or precursor tumors, **as active surveillance**, which was helpful to reduce work overload to the medical staff at the same time."

Page 17, in the discussion section, " **The third contributing factor might be** as 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 **those in other hospitals in a community hospital setting**."