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Original Article

Diagnostic performance of combination of ultrasound elastography and BRAF gene detection in malignant thyroid nodule: a retrospective study

Yuguo Wang[1], Xinping Wu[1], Jie Li[1], Jing Chen[1], Huafeng Tan[2], Liang Sun[3], Min Lu[4]

[1]*Department of Ultrasound, Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210028, China; Departments of [2]General Surgery, [3]Ultrasound, [4]Digestive, Nanjing Lishui District Hospital of Traditional Chinese Medicine, Nanjing 211200, China*

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Abstract: Objectives: The aims of our study were to explore the preoperative diagnostic value of ultrasound elastography combined with BRAF gene detection in malignant thyroid nodule, and find whether shear wave elastography (SWE) combined with BRAF gene detection can improve the diagnostic sensitivity and specificity. Methods: From 1480 patients with thyroid nodule examined between January 2015 and December 2017, a retrospective analysis was performed on 161 patients who underwent thyroidectomy. Diagnosis was confirmed by postoperative pathology, including 139 malignant thyroid nodules and 22 benign thyroid nodules. All the patients underwent SWE, BRAF gene detection, and the combination for their preoperative evaluation. The sensitivities, specificities, and accuracies of SWE, BRAF gene detection, and the combination for detection of malignant thyroid nodules were calculated and then compared using Fisher's exact probability test, based on the original preoperative reports and postoperative pathology. A receiver-operating characteristic curve analysis was performed to compare the diagnostic performance of SWE, BRAF gene detection, and combination for detecting malignant thyroid nodules. Results: Based on the original preoperative reports and postoperative pathology, SWE, BRAF gene detection, and the combination showed sensitivities of 88.67%, 78.41%, 92.8%, and specificities of 72.77%, 77.27%, 95.45%. A correct diagnosis was obtained in 85.82%, 78.26%, 93.16% and missed diagnosis rates were 12.23%, 21.58%, and 7.19%. The sensitivities, specificities, and correct diagnosis rate in the combination group were significantly higher than any single detection method (P<0.05). The missed diagnosis rate in the combination group was significantly lower than any single detection method (P<0.05). The receptor operating characteristics curve analysis showed a significantly higher diagnostic performance for the combination than for SWE and BRAF gene detection (P<0.05). The interobserver agreement for detecting malignant thyroid nodule was better for the combination than for SWE or BRAF gene detection alone. Conclusion: For the detection of a malignant thyroid nodule, SWE combined with BRAF gene detection was more sensitive and showed a higher diagnostic performance than SWE or BRAF gene detection alone.

Keywords: Ultrasound elastography, SWE, BRAF gene, malignant thyroid nodule

Introduction

Thyroid nodule disease is very common in many populations, and it was reported that the incidence for younger than 65 years was 33%, and for older than 65 years it was 50% [1]. In recent years, the incidence of thyroid nodular disease has increased, including adolescents and young adults [2]. The most important purpose of diagnostic testing is to evaluate the risk of malignancy in these nodules. The present revised American Thyroid Association management guidelines indicate that thyroid nodule detection rates are 5% by palpation, 19% to 67% by ultrasound (US), and 50% by autopsy [3]. The majority of the nodules are benign, and Hegedus [4] reported that 5% to 15% are malignant. Therefore, how to differentiate between malignant versus benign nodules on initial evaluation is very important for thyroid nodule management and treatment.

The optimal treatment for malignant thyroid nodules is surgical resection, and the sooner the operation is performed the better. Thus, early differentiation between malignant versus benign nodules is very important. As thyroid malignancies are characterized by insidious onset and variable biologic characteristics, the clinical, radiologic, and cytologic features are similar to benign nodules. Therefore, the preoperative misdiagnosis rate of malignant versus benign is high. At present, the differentiation methods are mainly thyroid ultrasound (US) and fine-needle aspiration biopsy (FNAB) cytology [5]. US and FNAB are very useful modalities for determining thyroid nodules, and the first-line and most preferred examination. However, many previous studies found that a large number of US images overlap between benign and malignant thyroid nodules, so this distinction by US alone is difficult [6]. Although FNA biopsy is the standard procedure to discriminate between malignant and benign thyroid nodules, it can definitively classify and with high sensitivity and specificity [7]. However, FNA is an invasive method, and the diagnostic performance/accuracy is based on the operator's experience. Indeterminate and nondiagnostic rates are as high as 15-30% [8]. Hence, we need a high sensitivity, specificity, and accuracy method for diagnosing malignant thyroid nodules.

Shear wave elastography (SWE) is a new noninvasive technology that can improve the sensitivity of thyroid cancer detection by measuring the degree of distortion [9]. This technique is important, less operator-dependent, gives more valuable information on tissue elasticity, and is more quantitative and reproducible than other techniques of elastography [10]. Many recent studies had demonstrated that SWE has the important ability to differentiate benign from malignant thyroid nodules [11], and it also was recommended in the international guidelines published by WFUMB [12]. However, it remains unclear whether SWE can accurately diagnose malignant thyroid nodules, as the results were inconsistent regarding variances in population and sample size.

2015 American Thyroid Association management guidelines recommend molecular testing for indeterminate nodules [5]. Molecular genetic testing can further verify the malignancy for indeterminate nodules, and reduce unnecessary surgery for negative molecular results. Research has demonstrated that gene expression classifiers can be used to distinguish which indeterminate nodules are malignant [13, 14]. Recent studies also indicated that BRAF mutation is better to differentiate between malignant versus benign nodules, and associated with aggressive-behaving thyroid malignancies [13, 15]. Even though previous studies indicated that the BRAF mutation is associated with papillary thyroid carcinoma, it is not sensitive for other malignant tumors in a thyroid nodule [16].

Thus, the objective of the present retrospective study was to evaluate the diagnostic properties of SWE combined with BRAF gene detection in the preoperative management. and sensitivities, specificities, and accuracies for a malignant thyroid nodule as verified histologically versus benign thyroid nodules.

Materials and methods

Patient population

We conducted a retrospective cohort study between January 2015 and December 2017 in the Department of Head and Neck Surgery, Nanjing Medical University Hospital, China. All consecutive patients underwent neck sonography using SWE and BRAF gene detection before surgical removal. All patients underwent thyroid surgery, providing histologic results of the specimens (100%). The average duration of disease was 15.8 months (range 6 weeks to 6.5 years). The inclusion criteria were as follows: (1) maximum diameter of the nodule was equal to or larger than 5 mm, and (2) BRAF gene detection and SWE results of nodules were complete. The exclusion criteria: (1) unavailable for SWE imaging or BRAF gene detection, (2) non-thyroid lesions such as almost cystic nodules, parotid gland, and cervical lymph node, (3) no histologic results of the specimens, and (4) not enough thyroid tissue surrounding the nodule (**Figure 1**).

SWE examination and ElastograPhy Score

Ultrasonography and SWE were performed preoperatively using the SuperSonic Aixplorer and a linear probe (4-9 MHz). All examination results were obtained by two radiologists (YMS and MS) blinded to the result of histopathology and who had more than 10 years of experience in BRAF gene detection by thyroid US and 5 years of elastography experience. All patients

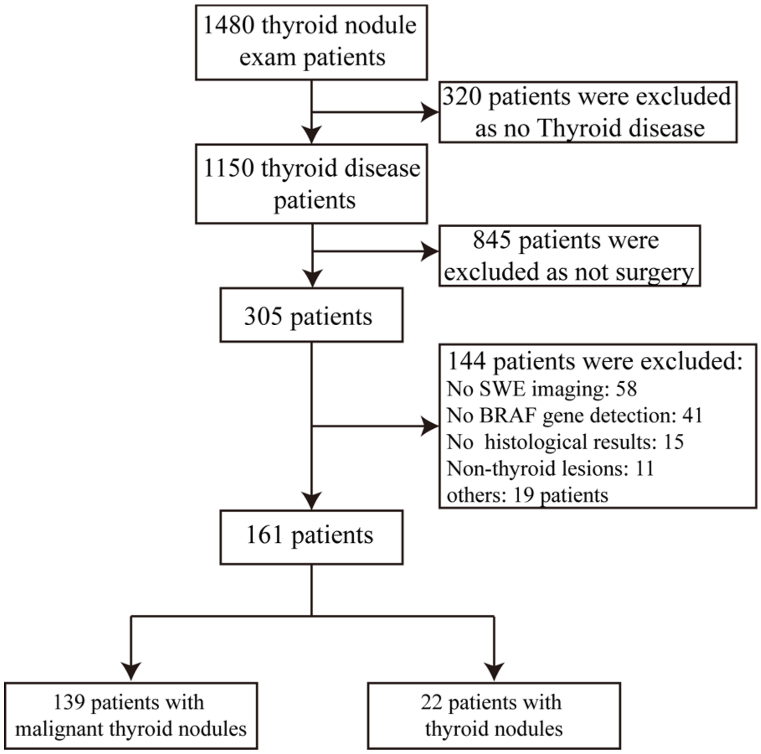

Figure 1. CONSORT diagram of the study.

were lying in a supine position with the neck slightly extended to explore the nodule better. Thyroid morphology, size, and boundary conditions were recorded, and the thyroid nodule size, boundary, internal echo, calcification, aspect ratio, color blood flow, and Elastography Score (ES). were evaluated carefully. Transverse and longitudinal sectional elasticity diagrams were performed for each thyroid nodule by ES, which were classified from 1 to 3, based on the degree of strain in the hypoechoic lesion. The scoring criteria were as follows: (1) score 0: the entire lesion was cystic with virtually nonsolid components, depicted as red, blue, and green mixture; (2) score 1: the lesion had a green area over the entire lesion; (3) score 2: Most of the lesion was green (green area > 50%); (4) score 3: The blue color was dominant in the lesional area (the blue area was 50%-90%); (5) score 4: The lesion was almost covered in blue (blue area > 90%) [17]. The higher ES and hardness of a thyroid nodule predicted a higher level of malignancy. Mostly, ES greater or equal to 3 points indicated malignant nodules, and ES less than 3 points indicated benign nodules.

BRAF gene detection

FNA was performed under ultrasound guidance, using very fine needles (25G/27G), without aspiration. FNA samples were smeared, Diff-Quik-stained, and evaluated on-site. DNA samples were subjected to BRAF mutational analysis utilizing the BRAF Codon 600 Mutation Analysis Kit II. The specific operation flow followed the manufacturer's procedures.

Statistical analysis

All data were analyzed using SPSS version 22.0 (SPSS, Chicago, IL, USA). Sensitivity was = a/(a+c)*100%, Specificity = d/(b+d)*100%, Positive predictive value (PPV) = a/(a+b)*100%, Negative predictive value (NPV) = d/(c+d)*100%, Misdiagnosis rate = b/(b+d)*100%, Missed diagnosed rate = c/(a+c)*100%. a was true positive, b was false positive, c was false negative, and d was true negative. Qualitative variables were presented in the form of frequencies and percentages. Categorical variables were analyzed by χ^2-test and Fisher's exact test was performed for the nominal variables. We determined the sensitivity, specificity, PPV, and NPV of SWE imaging or BRAF gene detection taking pathologic results as the gold standard. The optimal cutoff for differentiating benign nodules from malignant ones was determined using the receiver operating characteristic (ROC) curve. A two-tailed *P* value of <0.05 indicated significance.

Results

In this study, a total of 161 neck lesions from 161 patients were included. 38 patients were men and 123 were women, and their mean age was 42.5±14.1 years (range, 31-76 years). None of them had previous thyroid US and BRAF gene detection. All patients received ultrasonography, SWE, and BRAF gene detection before the operation. All patients had thyroid surgery with malignant or benign thyroid

Table 1. Results of SWE, BRAF, a combination, and postoperative pathologic results

Pathologic results	Case	SWE		BRAF		Combination	
		Positive	Negative	Positive	Negative	Positive	Negative
positive	139	122	17	109	30	129	10
negative	22	6	16	5	17	1	21
total	161	128	33	114	47	130	31

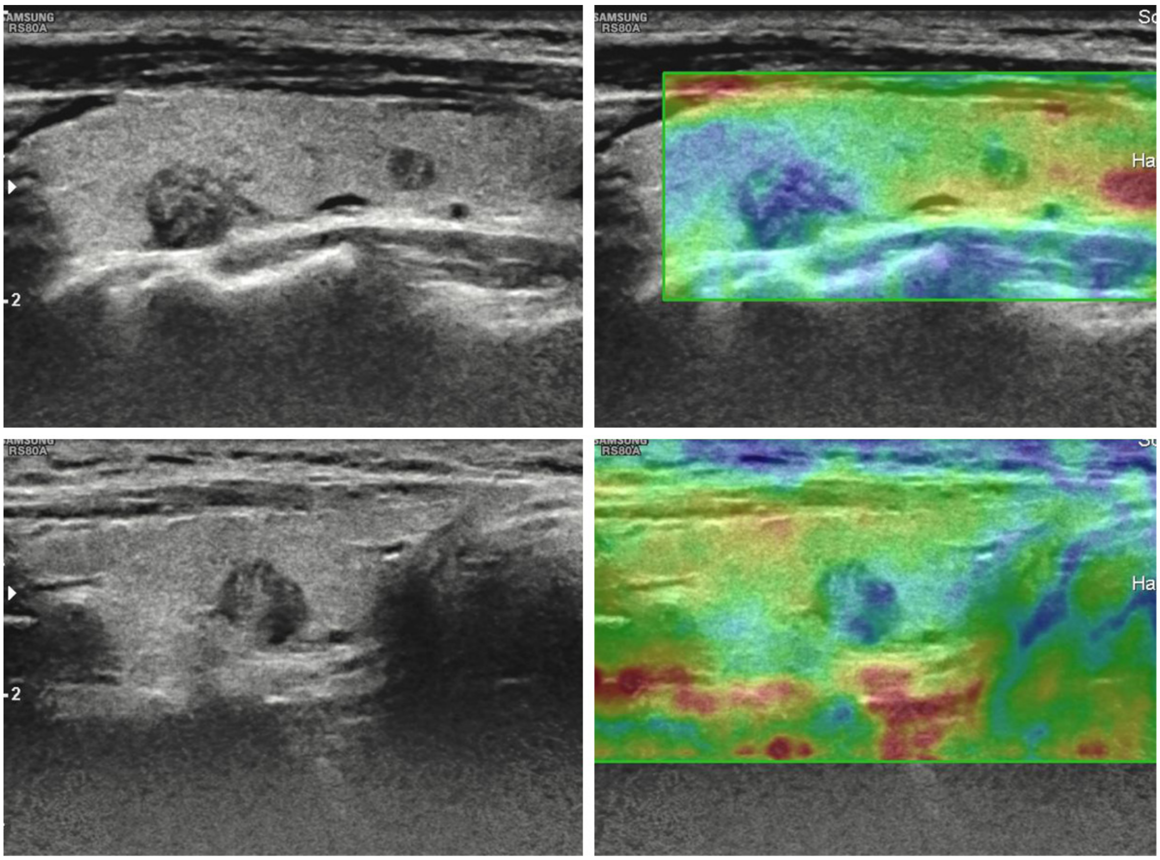

Figure 2. Images in a 47-year-old woman who underwent routine check-up. A right thyroid nodule with an irregular margin, hypoechogenicity, solid, with no calcification was found by grayscale US and assessed as a malignant nodule. The score of elastography was 3. The result of BRAF gene detection was malignant nodule after FNA. This thyroid nodule was diagnosed as papillary thyroid carcinoma at post-operative pathologic examination.

nodules confirmed pathologically, including 139 malignant thyroid nodules and 22 thyroid nodules.

In the present study, the postoperative pathologic examination confirmed 139 malignant thyroid nodules. 122 patients had a correct diagnosis by SWE examination, 17 patients had missed diagnosis. 109 patients had correct diagnosis by BRAF gene detection, 17 patients had missed diagnosis. 129 patients had a correct diagnosis by SWE examination combined with BRAF gene detection, 10 patients had missed diagnosis. Postoperative pathologic examination confirmed 22 benign thyroid nodules. 16 patients had correct diagnosis by SWE examination, 6 patients had misdiagnosis. 17 patients had correct diagnosis by BRAF gene detection, 5 patients had misdiagnosis. 21 patients had correct diagnosis by SWE examination combined with BRAF gene detection, 1 patient had misdiagnosis (**Table 1**). Typical cases can be seen (in **Figures 2-5**).

The sensitivity of SWE was 87.76%, specificity was 72.72%, PPV was 95.31%, NPV was 48.48%. The correct diagnosis rate was 85.71%, misdiagnosis rate was 27.27%, and

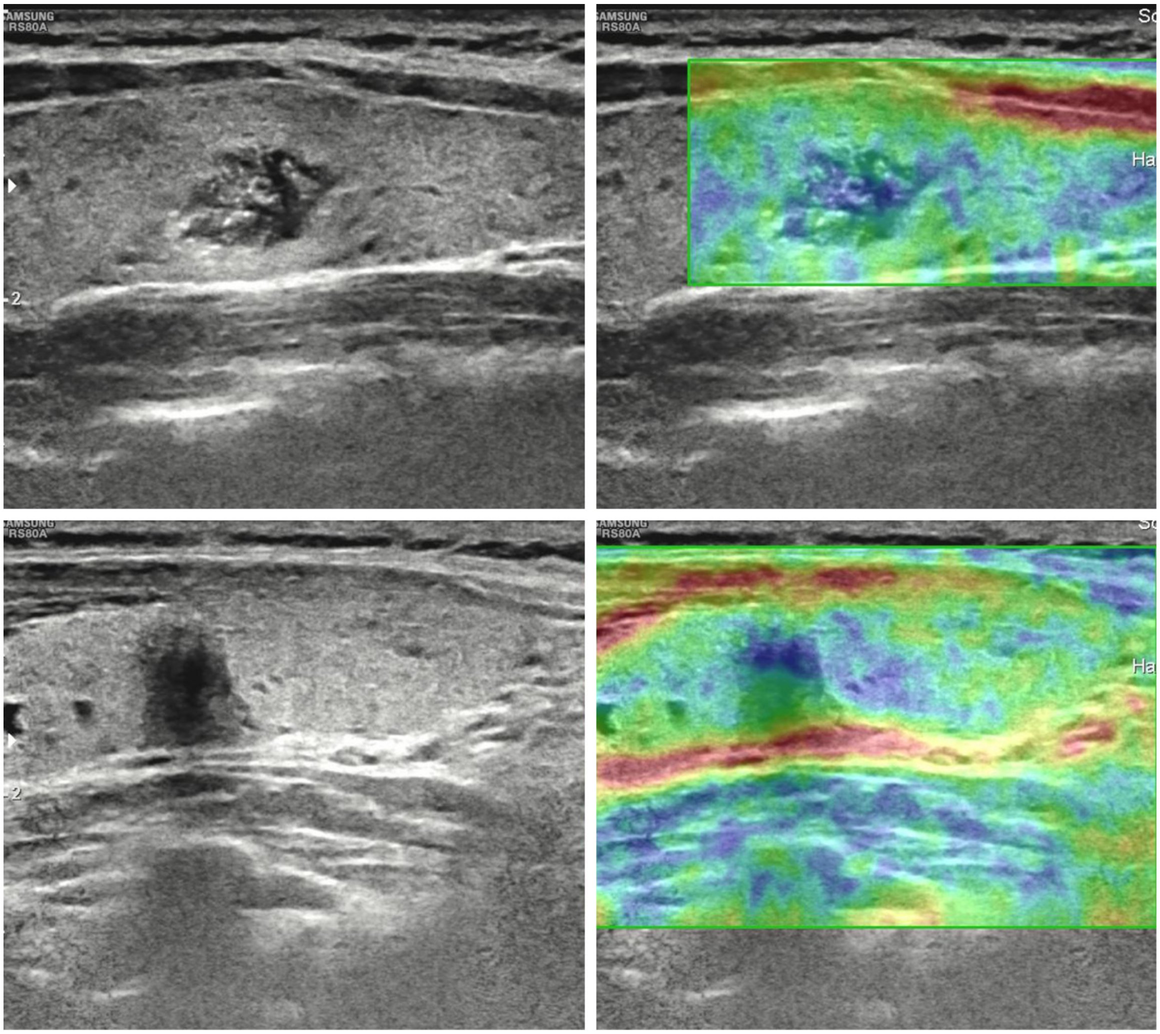

Figure 3. Images in a 35-year-old man who underwent routine check-up. A bilateral thyroid nodule with an irregular margin, hypoechogenicity, solid, and calcification was found by grayscale US and assessed as a malignant nodule. The score of elastography was 3. The result of BRAF gene detection was benign nodule after FNA. This thyroid nodule was diagnosed as benign nodule at post-operative pathologic examination.

missed diagnosis rate was 12.23%. The sensitivity of BRAF gene detection was 78.41%, specificity was 77.27%, PPV was 95.61%, and NPV was 36.17%. Correct diagnosis rate was 78.26%, misdiagnosis rate was 22.72%, and missed diagnosis rate was 21.58%. The sensitivity of SWE combined with BRAF gene detection was 92.8%, specificity was 95.45%, PPV was 99.23%, and NPV was 67.74%. The correct diagnosis rate was 93.16%, misdiagnosis rate was 4.5%, and missed diagnosis rate was 7.19%. The sensitivity, specificity, PPV, NPV, and correct diagnosis rate were higher than for BRAF gene detection or SWE. The misdiagnosis rate and missed diagnosis rate were reduced in the combination group versus any single examination method. The differences in sensitivity, NPV, and missed diagnosis rate were statistically significant ($P<0.05$) (**Table 2**).

In the distinction of malignancy, the AUC for the SWE was found to be 0.829 (95% CI: 0.73, 0.927), and the cutoff point was greater than 3. For the BRAF gene detection, the AUC was found to be 0.778 (95% CI: 0.683, 0.874). For the SWE combined with BRAF gene detection, the AUC was found to be 0.941 (95% CI: 0.892, 0.991). When we compared the AUC for three methods, the difference was significant, and this difference was statistically significant (for all, $P<0.01$). The diagnostic accuracy of the combination was superior to that of the SWE or BRAF gene detection (**Figure 6**).

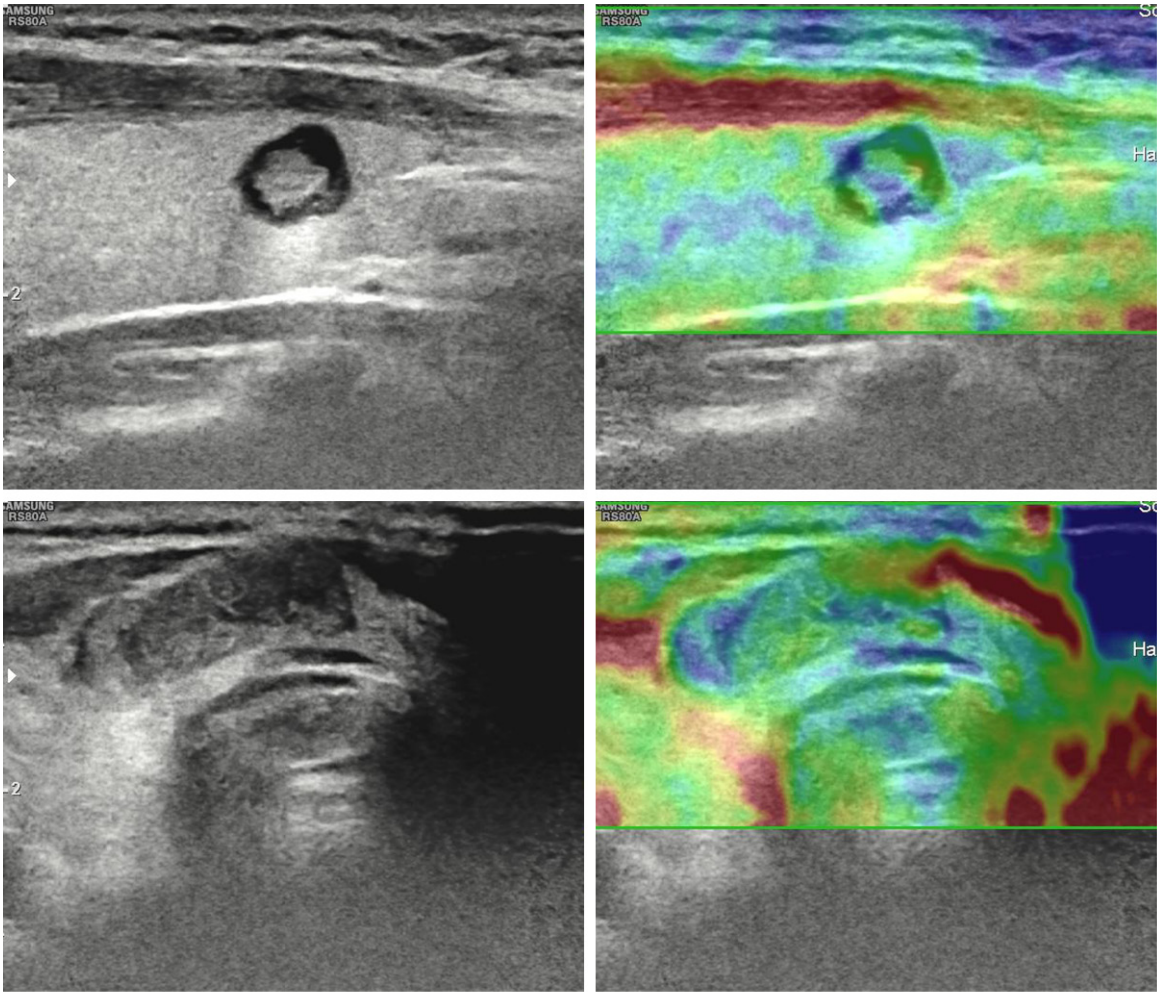

Figure 4. Images in a 41-year-old woman who underwent routine check-up. A right thyroid nodule with characteristics of a regular margin, hypoechogenicity, and cystic and solid nodule was found by grayscale US and assessed as a benign nodule. The score of elastography was 2. The result of BRAF gene detection was malignant nodule after FNA. This thyroid nodule was diagnosed as benign at post-operative pathologic examination.

Discussion

This single-center retrospective study found that the conventional US can detect nodules accurately but cannot differentiate malignant from benign nodules. Although thyroid FNA is the most sensitive and specific method for preoperative diagnosis of thyroid malignancies currently, about 0.7% to 15% of thyroid nodules cannot be diagnosed accurately by FNA [18]. In the present study, we confirmed that the sensitivity of BRAF gene detection after FNAC was 78.41%, specificity was 77.27%, PPV was 95.61%, and NPV was 36.17%. Correct diagnosis rate was just 78.26%, misdiagnosis rate as high as 22.72%, and missed diagnosis rate was 21.58%. Besides, the limitations of FNA were the surgical experience of the surgeon, sample size, and sample being indeterminate. Nondiagnostic rate was 15-30% by FNA alone. Dong [19] confirmed that very small thyroid nodules (≤5 mm) may easily cause false-positive FNAB results, while big nodules (> 20 mm) tend to produce false-negative FNAB results.

As described above, more than 30% of FNAB samples were indeterminate [8, 20], after which most patients underwent diagnostic thyroid surgery as nondiagnostic nodules [21]. This is also increased the number of unnecessary thyroidectomies. Therefore, additional methods or techniques to enhance the sensitivity and specificity of the FNAB examination are

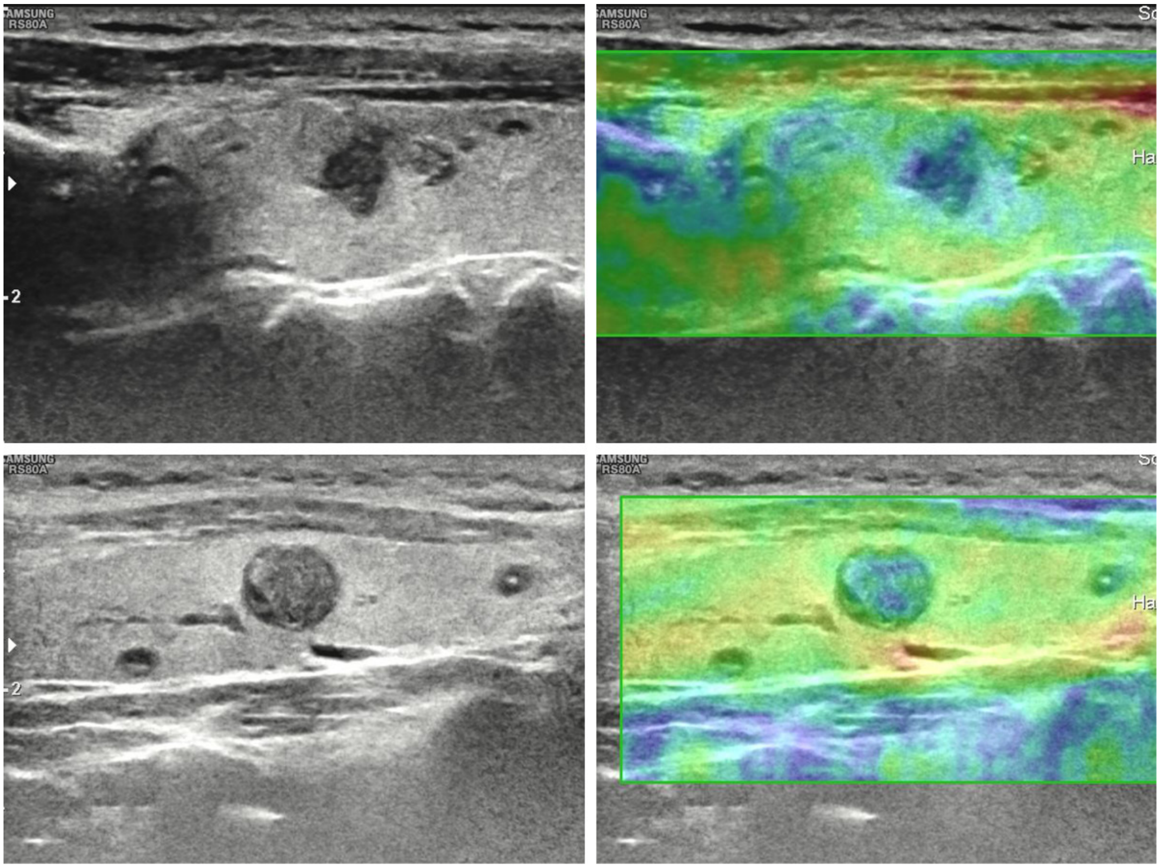

Figure 5. Images in a 29-year-old woman who underwent routine check-up. A right thyroid nodule with characteristics of a regular margin, hypoechogenicity, and cystic and solid nodule was found by grayscale US and assessed as a benign nodule. The score of elastography was 1. The result of BRAF gene detection was benign after FNA. This thyroid nodule was diagnosed as benign nodule at post-operative pathologic examination.

necessary, urgently needed, and important for clinical therapy. Molecular analysis of FNA samples is an effective auxiliary examination with high specificity and positive predictive value [22]. Recent studies had demonstrated that gene expression classifiers and gene mutation status may help us to differentiate the benign thyroid nodules from malignant thyroid nodules and guide the extent of initial surgery [21, 23]. Many studies indicated that BRAF V600E mutation was associated with papillary thyroid cancer (PTC), and this also showed the aggressive tumor behavior and high risk of malignancy [13]. Khatami [13] demonstrated that BRAF V600E mutation can best distinguish between PTC and thyroid nodules. PTC accounts for 60% of thyroid malignancies. BRAF mutation has a high detection rate in PTC, about 29%~84%, while it is not expressed in a benign thyroid nodule, and is also rarely expressed in other pathologic types [24]. The mechanism by which BRAF mutation enhances the aggression of PTC may be by activating the MAPK signaling pathway [15]. In the present study, we also found that BRAF gene detection was helpful in differential diagnosis and in increasing the efficiency of FNAB. Biron [25] reported a prospective study that enrolled 208 patients who underwent FNAB and mutational testing. Results showed that malignant cytology or BRAFV600E were 100% specific for malignancy, with PPV as high as 100%, and NPV was 89.7% for thyroid malignancy for BRAF V600E. Combined with the Bethesda system, BRAF V600E mutation examination can enhance the sensitivity and specificity of thyroid FNAB.

SWE was developed to noninvasively evaluate the tissue stiffness and strain. The main advantage of SWE is being noninvasive, easy to operate, and suitable for routine US examina-

Table 2. Diagnostic efficiency of SWE, BRAF gene detection, and the combination

Group	Sensitivity	Specificity	PPV	NPV	Correct diagnosis	Misdiagnosis	Missed diagnosis
SWE	87.76% (122/139)	72.72% (16/22)	95.31% (122/128)	48.48% (16/22)	85.82% (128/161)	28.57% (6/22)	12.23% (17/139)
BRAF	78.41% (109/139)	77.27% (17/22)	95.61% (109/114)	36.17% (17/47)	78.26% (114/161)	22.72% (5/22)	21.58% (30/139)
Combination	92.80% (129/139)	95.45% (21/22)	99.23% (129/130)	67.74% (21/31)	93.16% (130/161)	4.5% (1/22)	7.19% (10/139)
λ	12.66	4.28	3.88	11.48	5.33	4.28	12.56
P	0.002	0.118	0.144	0.003	0.069	0.118	0.002

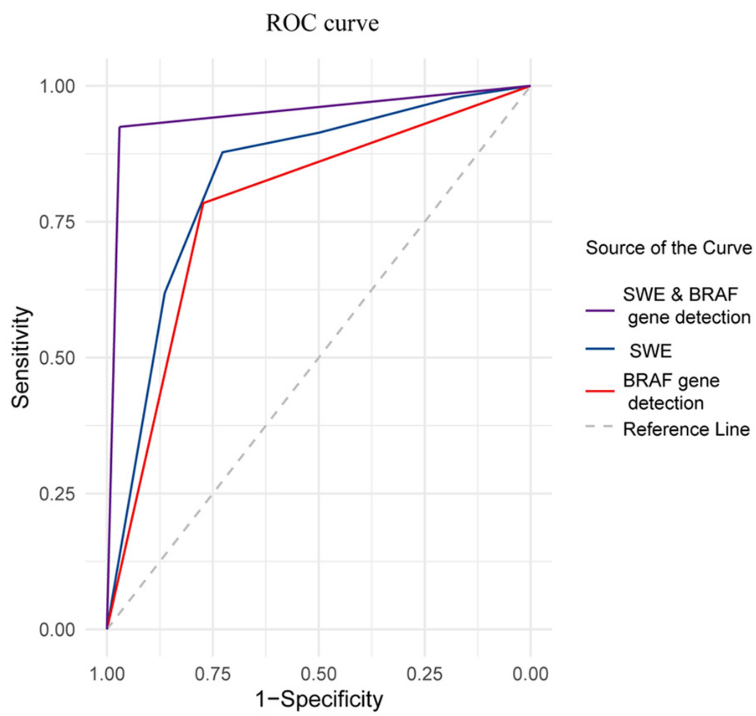

Figure 6. Receiver operating characteristic curves for distinguishing malignant micronodules from benign ones. SWE combined with BRAF gene detection (AUC = 0.941, 95% CI: 0.892, 0.991) contains the greatest area under the ROC curve. It was statistically significantly different from SWE (AUC = 0.829, 95% CI: 0.73, 0.927) or BRAF gene detection (AUC = 0.778, 95% CI: 0.683, 0.874) alone. ROC, receiver operating characteristic; AUC, area under the curve.

tions. In this study, all patients underwent SWE examination before FNA. The sensitivity of SWE was 87.76%, specificity was 72.72%, PPV was 95.31%, and NPV was 48.48%. The correct diagnosis rate was 85.71%, misdiagnosis rate was 27.27%, and missed diagnosis rate was 12.23%. The results of this study were similar to those of previous studies. Wang [26] reported that SWE was a very important method with a high sensitivity of 84.62%, a specificity of 78.45%, and an accuracy of 80.36% in thyroid nodules. Kagoya [27] also indicated that the sensitivity and specificity were 73% and 64% for predicting cancer, and 90% sensitivity and 50% specificity for a benign thyroid nodule. Many previous studies indicated that SWE was a useful technique for distinguishing malignancy, or that it could replace the FNAB or reduce the number of unnecessary FNABs [28]. Likewise in our study, we found that sensitivity and the correct diagnosis rate of SWE was higher than FNAB combined with BRAF gene detection, and missed diagnosis rate was lower.

SWE and BRAF gene detection are important diagnostic techniques for thyroid nodules and used for differential diagnosis of benign and malignant diseases with high sensitivity, specificity, and accuracy, while the missed diagnosis rate and misdiagnosis rate were high for a single examination technique alone. In this study, we also evaluated the sensitivity, specificity, correct diagnosis rate, missed diagnosis rate, and misdiagnosis rate by SWE techniques and BRAF gene detection before the operation. As we expected, the sensitivity, specificity, and correct diagnosis rate were enhanced significantly versus a single technique alone. The missed diagnosis rate and misdiagnosis rate in the combination group were decreased significantly compared to a single detection method. The diagnostic value of SWE technique combined with BRAF gene detection led to the highest accuracy, with an AUC of 0.985, a sensitivity of 92.80%, and a specificity of 95.45%. Hence, our results indicate that using both SWE technique and BRAF gene detection before the operation was a reliable method to differentiate between benign and malignant nodules. Similar studies are very rare. Jiang [29] and Hahn [30] also confirmed that SWE technology or BRAF gene detection alone could not be used as an independent diagnostic method. Many previous studies demonstrated that a combination of examination methods could improve the specificity and sensitivity in differentiating between benign and malignant thyroid nodules [31].

However, there were some limitations to our study. First, this was a retrospective analysis, lacking contrast. Secondly, the sample size of benign thyroid nodules was small, and we hope to increase the number of benign thyroid nodules sampled in future studies. In the future, a larger multicenter, randomized, controlled trial study is needed to evaluate combined use of ultrasound elastography and BRAF gene detection.

Conclusions

The application of SWE technology and BRAF gene detection may enhance the sensitivity, specificity, and correct diagnosis rate, and decrease the missed diagnosis rate and misdiagnosis rate. This approach also showed a higher diagnostic performance and may help to differentiate between benign and malignant thyroid nodules prior to operation, which can reduce unnecessary operations.

Disclosure of conflict of interest

None.

Address correspondence to: Min Lu, Department of Digestive, Nanjing Lishui District Hospital of Traditional Chinese Medicine, Nanjing 211200, China. E-mail: 3552601959@qq.com

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