Application of high-resolution ultrasound, real-time elastography, and contrast-enhanced ultrasound in differentiating solid thyroid nodules

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Abstract
High-resolution ultrasound (HRUS) is a sensitive tool for identifying thyroid nodules. Real-time elastography (RTE) and contrast-enhanced ultrasound (CEUS) are newly developed methods which could measure tissue elasticity and perfusion features. The aim of the present study was to evaluate and compare the diagnostic efficiency of HRUS, RTE, CEUS and their combined use in the differentiation of benign and malignant solid thyroid nodules.

In total, 111 consecutive patients with 145 thyroid nodules who were scheduled for surgery were included in the study. All of them underwent HRUS, RTE, and CEUS examination. The independent ultrasound (US) predictors for malignancy were determined and quantified using logistic regression analysis, based on which a risk-scoring model was established for each method. The diagnostic efficiency of each method was assessed by receiver operating characteristic (ROC) curve analysis.

HRUS showed the best diagnostic efficiency among the 3 US methods, with 74.6% sensitivity and 87.8% specificity. CEUS had higher sensitivity (85.7%), whereas RTE alone did not show much advantage. Combined use of RTE and HRUS increased the sensitivity (92.1%). The HRUS-RTE-CEUS combination could increase both the sensitivity and specificity (87.3%, 91.5%), with the best AUC (0.935) among all the methods.

The overall diagnostic value of HRUS in predicting malignancy is the best among the 3 US methods. Combined use of RTE and CEUS and HRUS could improve the diagnostic efficiency for solid thyroid nodules.

Abbreviations: ACQ = auto-tracking contrast quantification, APIFI = acoustic radiation force impulse, AUC = area under curve, CEUS = contrast-enhanced ultrasound, ES = elastography score, FNAC = fine-needle aspiration cytology, HRUS = high-resolution ultrasound, NPV = negative predictive value, PPV = positive predictive value, ROC = receiver operating characteristic, ROI = region-of-interest, RTE = real-time elastography, TIC = time-intensity curves, TI-RADS = thyroid imaging reporting and data system, TTP = time to peak, US = ultrasound.

Keywords: contrast-enhanced ultrasound, high-resolution ultrasound, real-time elastography, thyroid nodules

1. Introduction
Thyroid nodules are a common endocrine problem. They are discovered by palpation in 3% to 7% and by ultrasound (US) examination in 20% to 76% in the general population.[1] The incidence of thyroid cancer has increased over the past several decades[2-4] and is predicted to continue to increase between 50% and 60% by 2020.[4] In China, the annual report of Chinese Cancer Registry in 2009 showed that the incidence rate of thyroid cancer was 6.56 per 100,000, which ranked at 10 of the top types of cancer.[5] Although the majority of thyroid nodules are benign, differentiating malignancy from benign lesions is still the most challenging dilemma for clinicians.

High-resolution ultrasound (HRUS) is recommended as the first-line modality in the evaluation of thyroid nodules.[6] Solid composition, hypoechogenicity, microcalcification, irregular margin, taller than wide shape, and increased blood flow (centrally) are US findings associated with an increased risk for thyroid cancer.[7] However, the sensitivities and specificities for these criteria vary greatly in different studies. The combination of several suspicious US features is more accurate than that of any single characteristic in predicting a malignant thyroid nodule.[8,9] In 2009, Horvath et al.[10] and Park et al.[11] established a thyroid
imaging reporting and data system (TI-RADS) to stratify cancer risk based on 10 and 12 sonographic features respectively. A recent meta-analysis showed that TI-RADS had a pooled sensitivity and specificity of 0.75 and 0.69, respectively. However, there was a large range of sensitivity and specificity (0.37–0.96 and 0.43–0.94) with high heterogeneity probably due to different US equipment, different US criteria, as well as the inevitable inter-observer variability among radiologists.\textsuperscript{122}

Nowadays, new US technologies have been developed and applied to clinical practice enabling the determination of tissue elasticity and perfusion features. Real-time elastography (RTE), a reproducible assessment of tissue consistency,\textsuperscript{13} was reported to have a high sensitivity and specificity in the evaluation of thyroid nodules in a meta-analysis.\textsuperscript{114} Recently, contrast-enhanced ultrasound (CEUS), which could provide an assessment of perfusion over time, has been used for the differential diagnosis of thyroid nodules. Although CEUS was reported as a promising diagnostic method in a meta-analysis, it has not been widely used in clinical practice and its diagnostic value is still controversial.\textsuperscript{113,116}

To date, there were several papers that focused on the comparison of the overall diagnostic performance of RTE and CEUS.\textsuperscript{116–119} In Giusti’s study, RTE had a higher sensitivity, but greatly lower specificity than CEUS.\textsuperscript{116} However, Deng found no significant difference between RTE and CEUS.\textsuperscript{119} The CEUS parameters in these studies were not comprehensive and the applied statistical methods did not remove the confounding factors. In addition, cystic thyroid nodules were not suitable for RTE and CEUS evaluation. The aim of the present study was to evaluate and compare the diagnostic efficiency of HRUS, RTE, CEUS, and their combined use in the differentiation of benign and malignant solid thyroid nodules.

2. Methods

Between January 2012 and May 2015, 121 consecutive patients with 158 thyroid nodules were recruited for the study. These patients were recommended for surgery for at least one of the following criteria: (i) diagnosed as follicular neoplasm or suspicious for a follicular neoplasm, suspicious for malignancy or malignancy by fine-needle aspiration cytology (FNAC), (ii) the presence of BRAF\textsuperscript{V600E} mutation, (iii) compressive symptoms or cosmetic complaints, (iv) a significant increase in volume or a change in its US features during following up, (v) diagnosed as nondiagnostic or indeterminate lesions by FNAC, but showing 2 or more suspicious US criteria.\textsuperscript{16} The exclusion criteria were: (i) the presence of a typical nodular goiter or scintigraphically functional (“hot”) thyroid nodules, (ii) cystic nodules or nodules with egg-shell calcifications, (iii) incomplete elastography or time intensity curve data acquisition, (iv) any condition of hyperthyroidism, heart failure, or severe pulmonary hypertension, (v) previous adverse reaction to i.v. contrast agents. In the end, a total of 10 patients were excluded from the study based on the above criteria, and 111 patients with 145 nodules were included and evaluated. Their pathological types were confirmed by histologic analysis after surgery. Informed consent was obtained from all patients and the study was performed in accordance with the ethical guidelines of the Helsinki Declaration and approved by the first affiliated hospital with Nanjing Medical University ethics review committee (2012-SR-058).

This was a prospective study. All of the patients underwent HRUS (containing color-Doppler ultrasound), followed by RTE and CEUS using a Mylab Twice ultrasound unit (The Esaote Group, Genova, Italy). HRUS and RTE were performed with a LA523 transducer (4–13 MHz), whereas CEUS was performed with a LA522 transducer (3–9 MHz). All the examinations were performed by 2 radiologists repeatedly and the final decision was reached by a consensus. The radiologists were blind to the FNAC results. Using HRUS, the following sonographic features were assessed for each nodule: border (well-defined/ill-defined), shape (regular/irregular), echogenicity (isoechogenic/hyperechoic/ hypoechoic), echotexture (solid/predominantly solid/predominantly cystic/cystic/spongiform appearance), taller-than-wide (<1≥1), calcifications (absent/macrocalcification/microcalcification), halo sign (absent/present), and distal echo attenuation (absent/present).\textsuperscript{10} Borders were classified as well-defined (nodular surface was smooth and clearly distinguishable from the surrounding tissues) or ill-defined (the boundaries between the nodule and surrounding tissues were obscure).\textsuperscript{20} When the echogenicity of the nodule was similar to the surrounding thyroid parenchyma, it was classified as isoechogenicity. Echotexture was defined as the ratio of the cystic portion to the solid portion in the nodule: solid (≤10% cystic), predominantly solid (>10% cystic and ≤50% cystic), predominantly cystic (>50% cystic), and spongiform appearance. Taller-than-wide shape was defined as height divided by width on transverse views.\textsuperscript{21} Microcalcifications were defined as calcifications that were ≤1 mm in diameter, which was visualized as tiny, punctate hyperechoic foci, either with or without acoustic shadows. Macrocalcifications were defined as hyperechoic foci larger than 1 mm, including rim or egg-shell calcifications.\textsuperscript{22} Presence of halo sign was defined as the transonic rim surrounding lesion.\textsuperscript{23} Vascularization of thyroid nodules was classified using 3 different patterns: pattern I: no visible flow or minimal peripheral flow; pattern II: peripheral ring of flow but minimal or no internal flow, pattern III: extensive or moderate amount of internal flow with or without a peripheral ring.\textsuperscript{9}

RTE was applied by the ElaXto-Elastosonography technique. Elastogram was displayed over the B-mode image on a color scale that ranges from green (soft) to red (hard). Elastography score (ES) and ELX1/2 were assessed for each nodule. The scoring system was similar to the methods described by Asteria et al.:\textsuperscript{24} ES 1: homogeneously green; ES 2: predominantly green with few red areas/spots; ES 3: predominantly red with few green areas/ spots; ES 4: completely red. ELX1/2, the ratio between the elasticity features of the selected region-of-interest (ROI) located on the nodule and the ROI of US-normal thyroid tissue, was reported by the ElaXto software.

CEUS was performed with contrast tuned imaging (CnTi\textsuperscript{Tm}) software. SonoVue, a contrast agent, was injected as an intravenous bolus of 1.6 mL via a 20-gauge syringe into an antecubital vein, followed by a 5 mL saline flush. The thyroid gland including the nodule was scanned for about 4 minutes after bolus injection. The video clip was digitally recorded and further analyzed by auto-tracking contrast quantification (ACQ) software. Time-intensity curves (TIC) within selected ROI (in both nodules and the surrounding thyroid tissue) were acquired. Parameters of TIC: time to peak (TTP), peak intensity, sharpness, and area under curve were obtained by the ACQ software. The parameter in the nodule was divided by the same parameter in the surrounding thyroid tissue to avoid the individual difference.\textsuperscript{15} Time of contrast wash-in and wash-out of thyroid nodules were recorded as compared with the surrounding tissues. Types of contrast enhancement distribution were judged as homogeneous, heterogeneous, ring enhancement, and the differences of the intensity between the nodule and the periphery thyroid tissue were described as hypo-/equal-hyper-perfusion, or ring enhancement.\textsuperscript{19,25} In addition, characters of the contrast-enhanced area,
Table 1
Clinical features of the study population and basic characters of the nodules.

| Feature          | Benign | Malign | P   |
|------------------|--------|--------|-----|
| Sex              |        |        |     |
| Female           | 49 (86.0) | 42 (77.8) | 0.326 |
| Male             | 8 (14.0) | 12 (22.2) |       |
| Age, y           | 53.56 ± 10.97 | 42.19 ± 13.44 | <0.001 |
| Diameter, mm     | 16.73 ± 10.97 | 13.26 ± 8.44 | 0.043  |
| Multilocularity  |        |        | 0.006|
| Single           | 22 (38.6) | 30 (63.9) |       |
| Multiple (≥2)    | 35 (61.4) | 22 (36.1) |       |

border, shape were recorded as ≥50% or < 50%, well-defined or ill-defined, regular or irregular, respectively.

2.1. Statistical analysis

Statistical analysis was performed using the SPSS 13.0 software (SPSS Inc., Chicago). All quantitative values were expressed as means ± SD. Differences in the distribution of categorical variables between groups were evaluated by the 2-tailed Chi-square ($\chi^2$) test or Fisher exact test. The 2-tailed Student’s t-test was used to test for statistically significant differences in terms of distribution of quantitative variables between binary subgroups. A $P < 0.05$ was considered to be statistically significant. The receiver operating characteristic (ROC) curve analysis was used to identify the optimal cutoff of the quantitative variables according to the Youden index. A forward stepwise binary logistic regression analysis was performed to select independent predictors for malignancy from the US characteristics that showed statistical significance in univariate analysis, the entrance was 0.05, and exit $P$ value was 0.10. According to the $\beta$ coefficient of each variable obtained in the logistic regression analysis, a score for each significant predictive factor was assigned. For each nodule, the US risk score was obtained by a sum up of each individual score of ultrasound features. ROC curve analysis was performed to assess the diagnostic performance of the US risk score.

3. Results

3.1. Clinical profile

Totally 91 women and 20 men with 145 solid thyroid nodules were included in the study. Among the 145 nodules, 82 were benign and 63 malignant. Sixty-eight of the benign lesions were hyperplastic nodules and 14 were follicular adenomas. Of the malignant nodules, 60 were papillary carcinomas and 3 were follicular carcinomas. The clinical features of the study population and the basic characters of the nodules were showed in Table 1. Patients with malignant nodules were significantly younger than those with benign nodules ($P < 0.001$). Benign nodules had relatively larger diameters than malignant ones ($P < 0.05$). Compared with benign lesions, the risk of malignancy was higher in patients with a solitary nodule than with multiple nodules ($P < 0.05$).

3.2. High-resolution ultrasound (HRUS)

Among these analyzed features, ill-defined border, microcalcification, hypoechogenicity, irregular shape, and taller-than-wide shape showed a significant association with malignancy at univariate analysis (Table 2). The representative images of significant features were shown in Fig. 1. Logistic regression analysis revealed that the significant predictive variables were ill-defined border, microcalcifications, and hypoechogenicity (Table 2).

3.3. Real-time elastography (RTE)

The ELX1/2 showed no significant difference in benign and malignant nodules ($1.28 ± 0.35 vs 1.45 ± 0.56, P > 0.05$). ES1 was found in 8 nodules, all benign lesions; in nodules that scored ES 2, 3, and 4, the malignancy rates were 21.0%, 82.4%, and 80.0%, respectively. Image of ES 4 was shown in Fig. 1. The ES levels were significantly associated with malignancy ($P < 0.001$).

3.4. Contrast-enhanced ultrasound (CEUS)

First of all, quantitative analysis was performed based on the TIC (Fig. 2). Significant differences of TTP ratio, sharpness ratio, and peak ratio were found between benign and malignant nodules ($1.39 ± 0.95 vs 1.00 ± 0.43, 1.23 ± 0.60 vs 1.58 ± 0.91, 0.97 ± 0.38 vs 0.88 ± 0.43$, respectively, $P < 0.05$). The ROC curves were performed and revealed the optimal threshold for TTP ratio (1.15), sharpness ratio (1.60), and peak ratio (1.06) in the differentiation of benign and malignant solid thyroid nodules (Table 3). Perfusion patterns showed that fast wash-out was found more frequently in malignant nodules than benign ones ($P < 0.05$), whereas there was no significant difference in the time of wash-in between malignant and benign nodules ($P > 0.05$).

Table 2
Association between thyroid malignancy and various HRUS features on logistic regression analysis.

| Feature          | Benign | Malign | P   | $P/(95\% CI)$ | P  | Risk score |
|------------------|--------|--------|-----|---------------|----|------------|
| Border           |        |        |     |               |    |            |
| Well defined     | 75 (91.5) | 27 (42.9) | <0.001 | 2.127 (1.129-3.126) | <0.001 | 2.1 |
| Ill defined      | 7 (8.5)  | 36 (67.1) |       |               |    |            |
| Calcification    |        |        |     |               |    |            |
| Absent/macrocaltification | 73 (99.0) | 31 (49.2) | <0.001 | 1.556 (0.572-2.541) | 0.002 | 1.6 |
| Microcalcification | 9 (11.0)  | 32 (50.8) |       |               |    |            |
| Echogenicity     |        |        |     |               |    |            |
| Isoechoic/hyperechoic | 27 (32.9) | 2 (3.2)  |       |               |    |            |
| Hypoechogenicity | 55 (67.1) | 61 (96.8) | <0.001 | 2.322 (0.671-3.973) | 0.006 | 2.3 |
| Shape            |        |        |     |               |    |            |
| Regular          | 67 (81.7) | 29 (46.0) |       |               |    |            |
| Irregular        | 15 (18.3) | 34 (54.0) | <0.001 | 4.432 (1.671-11.330) | <0.001 |    |
| Taller-than wide |        |        |     |               |    |            |
| ≥1               | 78 (85.1) | 45 (71.4) |       |               |    |            |
|                 | 4 (4.9)  | 18 (28.6) | 0.001  |               |    |            |

HRUS = high-resolution ultrasound.
* Determined with the $\chi^2$ test.
† determined with logistic regression analysis.
Next, the qualitative perfusion features were analyzed. The ill-defined border, irregular shape, larger enhancement area in the contrast enhanced state and the perfusion intensity compared with normal thyroid tissue showed a significant association with malignancy (Table 3) (Fig. 3). No significant difference was found between malignant and benign nodules concerning types of contrast enhancement distribution. However, it was noted that

![Figure 1. HRUS images and RTE images: (A) ill-defined border; (B) irregular shape (C) microcalcifications; (D) taller-than-wide; (E) ES (4). ES = elastography score. HRUS = high-resolution ultrasound, RTE = real-time elastography.](image)

![Figure 2. Time intensity curve (TIC) of CEUS: (A) slow wash-in with slow wash-out; (B) rapid wash-in with slow wash-out; (C) rapid wash-in with rapid wash-out. Red line: normal thyroid tissue. Blue line: thyroid nodule. CEUS = contrast-enhanced ultrasound, TIC = time intensity curve.](image)

### Table 3

| Feature                      | Benign | Malign | P       | (95% CI) | P       | Risk score |
|------------------------------|--------|--------|---------|----------|---------|------------|
| TTP ratio ≥1.15              | 41 (50.0) | 13 (20.6) | 0.000 | 1.217 (0.292–2.142) | 0.010 | 1.2 |
| TTP ratio <1.15              | 41 (50.0) | 50 (79.4) |       |          |        |            |
| Sharpness ratio ≥1.60        | 68 (62.5) | 38 (60.3) | 0.004 |          |        |            |
| Sharpness ratio <1.60        | 14 (17.1) | 25 (39.7) |       |          |        |            |
| Peak ratio ≥1.06             | 33 (40.2) | 12 (19.0) | 0.007 |          |        |            |
| Peak ratio <1.06             | 48 (59.8) | 51 (81.0) |       |          |        |            |
| Enhancement border Well defined | 73 (89.0) | 26 (41.3) | <0.001 | 2.566 (1.638–3.494) | <0.001 | 2.6 |
| Enhancement border Ill defined | 9 (11.0)  | 37 (58.7) |       |          |        |            |
| Enhancement shape Regular    | 69 (84.1) | 26 (41.3) |       |          |        |            |
| Enhancement shape Irregular  | 13 (15.9) | 37 (58.7) | <0.001 |          |        |            |
| Perfusion intensity Ring-equal-hyper-perfusion | 62 (75.6) | 35 (55.6) |          |        |        |            |
| Perfusion intensity Hypo-perfusion | 25 (34.4) | 28 (44.4) | 0.013 |          |        |            |
| Enhanced area ≥50%           | 23 (28.0) | 8 (12.7)  | <0.001 | 2.566 (1.638–3.494) | <0.001 | 2.6 |
| Enhanced area <50%           | 59 (72.0) | 55 (87.3) | 0.040 |          |        |            |
| Time of wash-out Slow        | 54 (65.3) | 27 (42.9) | 0.007 | 0.836 (0.007–1.678) | 0.052 | 0.8 |

CEUS = contrast-enhanced ultrasound, TTP = time to peak.

* Determined with the \( \chi^2 \) test.

* Determined with logistic regression analysis.
the 5 nodules with ring enhancement were all proved to be benign.

The above 8 variables that showed significant difference in univariate analysis were included in logistic regression analysis. The result showed that the ill-defined enhancement border, fast wash out, and TTP ratio ≤1.15 were still significantly associated with malignancy (Table 3).

3.5. Evaluation of the diagnostic efficiency of different US methods

According to the ROC curve, the diagnostic value of HRUS was the greatest among the 3 US methods, with an AUC of 0.854. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 74.6%, 87.8%, 82.5%, and 81.8%, respectively (Table 4). As for elastography score, a presumptive malignant or benign diagnosis was assigned to ES3 and 4, ES1 and 2, respectively. The AUC was 0.804. RTE had relatively lower sensitivity (73.0%) and an equal specificity (87.8%) compared with HRUS (P = 1.000, P = 0.803) (Table 4). The ROC curve for CEUS showed that the AUC was 0.828. The specificity and PPV (68.3% and 67.5%) of CEUS were the lowest, whereas the sensitivity and NPV (85.7% and 86.2%) were the best among the 3 US methods Compared to HRUS, CEUS had a significantly lower specificity (68.3% vs 87.8%, P = 0.002) with a increase in sensitivity (85.7% vs 74.6%, P = 0.121) (Table 4). Then, the diagnostic performances of different combinations of US methods were evaluated. Logistic regression analysis was performed and risk-scoring models were built for combinations of HRUS-RTE, HRUS-CEUS, and HRUS-RTE-CEUS, respectively (Table 5). For HRUS-RTE, ill-defined border, microcalcifications, hypoechogenicity, and ES 3/4 have significant predictive value for malignancy (Table 5). The HRUS-RTE model showed increased AUC and sensitivity (0.917 vs 0.854, P = 0.003, 92.1% vs 74.6%, P = 0.003, respectively), with a slightly decrease in the specificity (81.7% vs 87.8%, P = 0.074) when compared with HRUS alone (Table 4).

For HRUS-CEUS, the result showed that ill-defined border, microcalcifications, hypoechogenicity, TTP ratio ≤1.15 were independent predictors for malignancy (Table 5). The HRUS-CEUS prediction model also showed slightly increased AUC (0.884), but sensitivity, specificity, PPV, NPV were the same as HRUS alone (74.6%, 87.8%, 82.5%, and 81.8%, respectively) (Table 4).

Regarding for HRUS-RTE-CEUS, the following US features showed significant predictive value for malignancy: ill-defined border, microcalcifications, hypoechogenicity, ES 3/4, TTP ratio <1.15, and peak ratio <1.06 (Table 5). Both the sensitivity (87.3%) and specificity (91.5%) of this prediction model were increased compared to HRUS alone, though, not significant (P = 0.061, P = 0.504), whereas AUC increased significantly (P = 0.001). When compared to HRUS-RTE, the sensitivity, specificity, and AUC of HRUS-RTE-CEUS showed no significant difference (P = 0.249, P = 0.133, P = 0.197). The difference between HRUS-CEUS and HRUS-RTE-CEUS in sensitivity and specificity was also not significant (P = 0.061, P = 0.505), whereas AUC was significantly different (P = 0.006) (Table 4). Figure 4 showed the ROC curves of the 3 US methods.

4. Discussion

In the present study, we performed a binary logistic regression analysis to identify the ultrasonic diagnostic criteria for solid neoplasms.

| Table 4 |

| Diagnostic accuracy of different ultrasound scores for malignant nodules. |
|----------|----------|----------|----------|----------|----------|
|          | AUC (95%CI) | Cutoff | Sensitivity | Specificity | PPV | NPV |
| HRUS     | 0.854 (0.786–0.907) | 3.0 | 74.6 | 87.8 | 82.5 | 81.8 |
| RTE      | 0.804 (0.767–0.906) | 3.0 | 73.0 | 87.8 | 82.1 | 80.9 |
| CEUS     | 0.828 (0.756–0.880) | 1.6 | 85.7 | 68.5 | 67.5 | 86.2 |
| HRUS-RTE | 0.917 (0.859–0.956) | 3.25 | 92.1 | 81.7 | 70.5 | 90.1 |
| HRUS-CEUS| 0.884 (0.821–0.931) | 4.25 | 74.6 | 87.8 | 82.5 | 81.8 |
| HRUS-RTE-CEUS | 0.935 (0.881–0.969) | 6.05 | 67.3 | 91.5 | 88.7 | 90.4 |

AUC = area under curve, CEUS = contrast-enhanced ultrasound, CI = confidence interval, HRUS = high-resolution ultrasound, NPV = negative predictive value, PPV = positive predictive value, RTE = real-time elastography.
thyroid nodules and evaluated the diagnostic performance of different ultrasound methods by a risk scoring model. We found that generally HRUS has the best diagnostic efficiency among the 3 US methods. CEUS has higher sensitivity, whereas HRUS and RTE showed equally high specificity. When combined with HRUS, CEUS did not enhance the diagnostic value like RTE did. The combination of HRUS-RTE-CEUS could increase both the sensitivity and specificity of ultrasound and provide the best diagnostic value for solid thyroid nodules.

HRUS is the most sensitive test to detect thyroid nodules and select the lesions for FNA biopsy. In the present study, we confirmed that the following features such as ill-defined border, microcalcification, hypoechogenicity, irregular shape, and taller-than-wide showed significant difference between benign and malignant solid nodules. However, only ill-defined border, microcalcification, and hypoechogenicity were independent predictors of malignancy on logistic regression analysis. We found that hypervascularity was not a sole predictor of malignancy with a sensitivity of 77.8% and a low specificity of 32.9%. According to the risk-scoring system based on each suspicious US feature, the AUC of ROC curve reached 0.854. Similar to our study, Kwak et al[22] and Cheng et al[23] also build prediction models to assess the diagnostic value of HRUS, finding that the sensitivity and specificity were 80.0% and 90.5%, the AUC reached 0.867. These findings were in accordance with the guidelines for the diagnosis and management of thyroid nodules made by AACE, AME, and ETA.5 Our data demonstrated that the overall diagnostic performance of HRUS was the best among 3 US methods, indicating that conventional HRUS is still the most important US tool in evaluating thyroid nodules.

RTE, the first commercially available elastographic imaging technique, has been widely applied in the diagnosis of thyroid nodules. The overall mean sensitivity and specificity for differentiation of thyroid nodules were 0.77 to 0.81 and 0.76 to 0.79 for elasticity score assessment and 0.81 to 0.89 and 0.77 to 0.83 for strain ratio assessment, respectively.[27] In the present study, we used a Mylab Twice ultrasound unit that could be able to measure tissue elasticity and display elastography score and quantitatively ELX 1/2. The ELX 1/2 showed no significant difference between benign and malignant nodules, just the same as Giusti’s results, but different from other previous reports.[18,28,29] However, the combination of ES 3 and ES 4 approached 73.0% sensitivity and 87.8% specificity in detecting thyroid cancer. The overall diagnostic value of RTE was lower than HRUS. The specificity of RTE recorded herein was higher than the meta-analysis results, but its sensitivity appeared slightly lower.[27] This distinction is probably due to different US equipment and the inevitable intra- and inter-observer variability both in the data acquisition and interpretation.[30] There are several limitations of RTE. Follicular carcinoma, thyroid nodules with coarse calcification, as well as cystic lesions are not suitable for RTE evaluation.[16,31,32] Our data showed that 2 of the 3 follicular carcinomas were missed by RTE. RTE showed a low specificity of 61.5% among the 17 nodules with macrocalcification, which was similar to Kim’s[32] results that elastography had only 51.1% specificity in calcified nodules.

The usage of CEUS in thyroid disease is restricted to definition of the size and limits of necrotic zones after US-guided ablation procedures.[31] Its value is still controversial due to the lack of unified criteria for diagnosis. In this study, we established the useful diagnostic criteria for thyroid nodules by evaluating both qualitative and quantitative CEUS features comprehensively. The results of univariate analysis showed that shorter time of wash-out, peak ratio <1.06, sharpness ratio <1.60, TTP ratio <1.15, ill-defined border, irregular shape and hypo-perfusion, and larger enhancement area were significantly related with malignancy. As for enhancement pattern, ring enhancement showed a 100% probability of benign. Our findings partly agreed with Deng’s[19]
results, which indicated that hypoenhancement was malignancy predictor, whereas ring enhancement only appeared in benign nodules. But we did not confirm the value of heterogeneous enhancement for predicting malignancy.\textsuperscript{117,211} For quantitative analysis, the 4 parameters of TIC in nodules per se did not show meaningful association between malignant and benign nodules. However, the peak ratio, TTP ratio, and sharpness ratio, after correction of the surrounding thyroid tissue, showed significant relation with histology. However, on binary logistic regression analysis, only rapid wash-out, ill-defined enhancement border, TTP ratio < 1.15 were independent predictors of malignancy. The sensitivity of HRUS in our study was similar to that in the meta-analysis (85.7\% vs 85.3\%), whereas the specificity was lower (68.3\% vs 87.6\%).\textsuperscript{131} Compared with HRUS and RTE, the sensitivity and NPV of CEUS were the highest, whereas the specificity and PPV were the lowest. It seems that CEUS alone had limited application value in differentiating malignant and benign thyroid nodules when compared with HRUS. This phenomenon may be partly ascribed to the complexity of microcirculation in tumor, unskilled operation, and lack of accepted standards or guidelines.\textsuperscript{149} However, we found that the 3 US methods were highly complementary in predicting malignancy. CEUS could help to find more malignant nodules missed by HRUS and RTE, which merely show as hypervascularity and hypoechochogenity in HRUS.

To further evaluate the diagnostic value of RTE and CEUS combined with HRUS, risk-scoring models were built for different combinations of US methods. For the combination of HRUS and RTE, ES 3/4, microcalciﬁcations, hypoechochogenicity, and ill-deﬁned border were independent predictors of malignancy. Our results support the conclusions of Azizi et al.\textsuperscript{131} who demonstrated that ES, microcalciﬁcations, hypoechochogenicity, and isthmus location were independent predictors of thyroid cancer. The sensitivity of US was increased when combined with RTE, which was partly in line with other authors who showed that combined use of both methods could raise the diagnostic sensitivity from 85\% to 97\%, 93.2\% to 96.7\%, respectively.\textsuperscript{136,37} Regarding the combination of HRUS and CEUS, the diagnostic performance of the HRUS-CEUS model did not show enhancement compared to that of HRUS alone, unlike the results in Deng’s study.\textsuperscript{119} However, the interpretation of conventional ultrasound and CEUS results in their study was based on experience or single characteristic. When HRUS, RTE, and CEUS were combined together, ill-deﬁned border, microcalciﬁcation, hypoechochogenicity, ES 3/4, TTP ratio < 1.15, peak ratio < 1.06 were independent predictors, which increased the sensitivity to 87.3\%. The AUC and speciﬁcity of HRUS-RTE-CEUS were the highest among all the US methods.

In 2014, Liang et al.\textsuperscript{220} performed binary logistic regression analysis to select predictors for malignancy and further analyzed the diagnostic value of HRUS, CEUS, and ARFI (Acoustic Radiation Force Impulse). The diagnostic performance was increased by the combination of the 3 methods with an AUC of 0.943, which is similar to our results. However, there were some differences between the 2 studies. (1) Elastography methods: Liang interpreted the elasticity using Acoustic Radiation Force Impulse, a second-generation elastography method, whereas we focused on the traditional real-time elastography. (2) CEUS: we analyzed all the quantitative and qualitative parameters of CEUS, more comprehensive than Liang’s study which just focused on 3 enhancement patterns, peak index, and TTP index. (3) Statistical method: though using binary logistic regression as well, Liang performed the regression analysis just in order to establish an equation to predict the malignancy risk of a nodule, and the comparison of the diagnostic value between the different combinations of the 3 methods was just based on univariate analysis results. In the present study, when comparing the diagnostic value of the 3 techniques, we also used forward stepwise binary logistic regression analysis to filter out the factors with real statistical signiﬁcance from multiple factors and built ROC curves to compare the AUC of different methods, which was more reasonable and convincing. Taking into consideration that each predictive factor may contribute to the final result to a different degree, we built the risk-scoring model to assess thyroid nodules. This method could avoid the downsides of parallel test or serial test when evaluating the diagnostic value of combinations of different methods, providing more accurate prediction. Our study demonstrated that although the diagnostic performance of RTE or CEUS alone was limited, RTE and CEUS could increase the sensitivity when combined with HRUS, indicating that the new US techniques were potentially useful tools in the workup of thyroid nodules.

The limitations of our study should also be addressed. First, our study population was constituted by patients already selected for surgery and the selecting bias was unavoidable. The frequency of malignancy was higher than expected in a general population of patients with thyroid nodules, which may cause the overestimation of PPV and underestimation of NPV.\textsuperscript{138} Second, benign nodules included in our study had relatively larger diameters than malignant nodules. The size differences might affect the evaluation of diagnostic efﬁciency among the 3 US methods. Our results need to be conﬁrmed with a prospective study on a nonsurgical population. Moreover, the sample size of the present study is not large enough, so veriﬁcation is needed in larger multicenter studies which would allow for more accurate calculation of sensitivity, speciﬁcity and predictive values.

5. Conclusions
The overall diagnostic value of HRUS in predicting malignancy is the best among the 3 US methods and ill-deﬁned border, microcalciﬁcation and hypoechoic were the most predictive features of HRUS. The diagnostic value of CEUS is second only to HRUS with the signiﬁcant parameters of TTP ratio < 1.15 and peak ratio < 1.06. ES 3/4 alone has the lowest diagnostic efﬁciency in the characterization of thyroid nodules. RTE could increase the sensitivity, whereas CEUS has little additional value when combined with HRUS. Combination of HRUS, RTE, and CEUS could increase both the diagnostic sensitivity and speciﬁcity compared to HRUS alone, which showed the best diagnostic efﬁciency. The use of RTE and CEUS might be considered in combination with HRUS to improve diagnostic value, although further studies will be needed to verify our predictive models and to standardize the usage of RTE and CEUS.

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References
[1] Garib H, Papini E, Valcavi R, et al. American association of clinical endocrinologists and associazione medici endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules. Endocr Pract 2006;12:63–102.
[1] Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973–2002. JAMA 2006;295:2164–7.
[2] Chen AY, Jemal A, Ward EM. Increasing incidence of differentiated thyroid cancer in the United States, 1988–2005. Cancer 2009;115:3801–7.
[3] Weir HK, Thompson TD, Soman A, et al. The past, present, and future of cancer incidence in the United States: 1975 through 2020. Cancer 2015;121:1827–37.
[4] Chen W, Zheng R, Zhang S, et al. The incidences and mortalities of major cancers in China, 2009. Chin J Cancer 2013;32:106–12.
[5] Horvath E, Majlis S, Rossi R, et al. An ultrasonogram reporting system. J Clin Endocrinol Metab 2012;97:4524–60.
[6] Park JY, Lee HJ, Jang HW, et al. A proposal for a thyroid imaging reporting and data system on 4550 nodules with and without malignancy. Radiology 2012;264:380–10.
[7] Brito JP, Gionfriddo MR, Al Nofal A, et al. The accuracy of thyroid nodule ultrasound to predict thyroid cancer: systematic review and meta-analysis. J Clin Endocrinol Metab 2014;99:1253–63.
[8] Moon WJ, Jung SL, Lee JH, et al. Benign and malignant thyroid nodules: US differentiation—multicenter retrospective study. Radiology 2008;247:762–70.
[9] Ozel A, Erturk SM, Erkan A, et al. The diagnostic efficiency of ultrasound in characterization for thyroid nodules: how many criteria are required to predict malignancy? Med Ultrason 2012;14:24–8.
[10] Horvath E, Majlis S, Rossi R, et al. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab 2009;94:1748–51.
[11] Park JY, Lee HJ, Jang HW, et al. A proposal for a thyroid imaging reporting and data system for ultrasound features of thyroid cancer. Thyroid 2009;19:1237–45.
[12] Wei X, Li Y, Zhang S, et al. Thyroid imaging reporting and data system (TI-RADS) in the diagnostic value of thyroid nodules: a systematic review. Tumour Biol 2014;35:6769–76.
[13] Rago T, Santini F, Scutari M, et al. Elastography: new developments in characterization for thyroid nodules: how many criteria are required to predict malignancy? Med Ultrason 2012;14:24–8.
[14] Boujena J, Herrmann E, Meyer G, et al. Real-time elastography for the differentiation of benign and malignant thyroid nodules: a meta-analysis. Thyroid 2010;20:1145–50.
[15] Bartolotta TV, Midiri M, Gialla M, et al. Qualitative and quantitative evaluation of solitary thyroid nodules with contrast-enhanced ultrasound: initial results. Eur Radiol 2006;16:2234–41.
[16] Friedrich-Rust M, Sperber A, Holzer K, et al. Real-time elastography and contrast-enhanced ultrasound for the evaluation of thyroid nodules. Exp Clin Endocrinol Diabetes 2010;118:602–9.
[17] Cantone V, Consorti F, Guerini A, et al. Prospective comparative evaluation of quantitative elastasonography (Q-elastography) and contrast-enhanced ultrasound for the evaluation of thyroid nodules: preliminary experience. Eur J Radiol 2013;82:1892–8.
[18] Giusti M, Orlando D, Melle G, et al. Is there a real diagnostic impact of elastasonography and contrast-enhanced ultrasound in the management of thyroid nodules? J Zhejiang Univ Sci B 2013;14:195–206.
[19] Deng J, Zhou P, Tian SM, et al. Comparison of diagnostic efficacy of contrast-enhanced ultrasound, acoustic radiation force impulse imaging, and their combined use in differentiating focal solid thyroid nodules. PLoS One 2014;9:e90674.
[20] Liang XN, Guo RJ, Li S, et al. Binary logistic regression analysis of solid thyroid nodules imaged by high-frequency ultrasound, acoustic radiation force impulse, and contrast-enhanced ultrasoundography. Eur Rev Med Pharmacol Sci 2014;18:3601–10.
[21] Shao J, Shen Y, Lu J, et al. Ultrasound scoring in combination with ultrasound elastography for differentiating benign and malignant thyroid nodules. Clin Endocrinol (Oxf) 2015;83:254–60.
[22] Kwak JY, Jung I, Baek JH, et al. Image reporting and characterization system for ultrasound features of thyroid nodules: multicentric Korean retrospective study. Korean J Radiol 2013;14:110–7.
[23] Appereccia M, Solvetti FM. The association of colour flow Doppler sonography and conventional ultrasonography improves the diagnosis of thyroid carcinoma. Horm Res 2006;66:249–56.
[24] Astoria C, Giovanardi A, Pizzocaro A, et al. US differentiation in the differential diagnosis of benign and malignant thyroid nodules. Thyroid 2008;18:523–31.
[25] Zhang B, Jiang YX, Liu JB, et al. Utility of contrast-enhanced ultrasound for evaluation of thyroid nodules. Thyroid 2010;20:51–7.
[26] Cheng PW, Chou HW, Wang CT, et al. Evaluation and development of a real-time predictive model for ultrasound investigation of malignant thyroid nodules. Eur Arch Otorhinolaryngol 2014;271:1199–206.
[27] Sun J, Cai J, Wang X. Real-time ultrasound elastography for differentiation of benign and malignant thyroid nodules: a meta-analysis. J Ultrasound Med 2014;33:591–5.
[28] Cahal S, Aydin C, Korukluoglu B, et al. Diagnostic value of elastographically determined strain index in the differential diagnosis of benign and malignant thyroid nodules. Endocrine 2011;39:89–98.
[29] Ding J, Cheng H, Ning C, et al. Quantitative measurement for thyroid cancer characterization based on elastography. J Ultrasound Med 2011;30:1259–66.
[30] Park SH, Kim SJ, Kim EK, et al. Interobserver agreement in assessing the sonographic and elastographic features of malignant thyroid nodules. AJR Am J Roentgenol 2009;193:W416–23.
[31] Tranquant F, Bleuzen A, Pierre-Renoult P, et al. Elastasonography of thyroid lesions. J Radiol 2008;89:55–9.
[32] Kim MH, Luo S, Ko SH, et al. Elastography can effectively decrease the number of fine-needle aspiration biopsies in patients with calcified thyroid nodules. Ultrasound Med Biol 2014;40:2329–35.
[33] Yu D, Han Y, Chen T. Contrast-enhanced ultrasound for differentiation of benign and malignant thyroid lesions: meta-analysis. Otolaryngol Head Neck Surg 2014;151:909–1015.
[34] Argalia G, De Bernardis S, Mariani D, et al. Ultrasonographic contrast agent: evaluation of time-intensity curves in the characterisation of solitary thyroid nodules. Radiol Med 2002;103:407–13.
[35] Azzini G, Keller J, Lewis M, et al. Performance of elastography for the evaluation of thyroid nodules: a prospective study. Thyroid 2013;23:734–40.
[36] Trimbioli P, Guglielmi R, Monti S, et al. Ultrasound sensitivity for thyroid malignancy is increased by real-time elastography: a prospective multicenter study. J Clin Endocrinol Metab 2012;97:4524–30.
[37] Russ G, Royer R, Rigorgne C, et al. Prospective evaluation of thyroid imaging reporting and data system on 4530 nodules with and without elastography. Eur J Endocrinol 2013;168:649–55.
[38] Ferris RL, Baloch Z, Bernet V, et al. American Thyroid Association Statement on Surgical Application of Molecular Profiling for Thyroid Nodules: Current Impact on Perioperative Decision Making. Thyroid 2015;25:760–8.