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HEAD AND NECK IMAGING

ORIGINAL ARTICLE

Evaluation of contrast-enhanced ultrasound-guided fine needle aspiration biopsy in thyroid nodule diagnosis: a study from two tertiary referral centers

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PURPOSE

This study aimed to compare the final definitive diagnosis (FDD) rate and accuracy of contrast-enhanced ultrasound (CEUS)-guided fine-needle aspiration biopsy (FNAB) with conventional US-guided FNAB.

METHODS

A two-center study was conducted to compare the diagnostic performance of FNAB with or without CEUS guiding between April 2014 and April 2024. In center 1, 1,074 patients with thyroid nodules who had undergone FNAB were retrospectively evaluated. Among them, 526 nodules were guided by CEUS, and 548 nodules were guided by conventional US. In center 2, 382 patients were included for a prospective study; 91 nodules received CEUS-guided FNAB and 291 nodules received conventional US-guided FNAB. The rate of non-diagnosis and indeterminate results of the two kinds of FNAB were calculated, and the FDD rate and diagnostic accuracy were compared in the two centers.

RESULTS

In the retrospective study, there were significant differences between FNABs guided by CEUS and those guided by conventional US in the non-diagnostic rates (0.4% vs. 6.2%, $P < 0.001$) and indeterminate rates (4.4% vs. 11.1%, $P < 0.001$); the rates of FDD were 93.3% vs. 77.9%, ($P < 0.001$), and the accuracy was 99.8% vs. 98.1 ($P = 0.015$). In the prospective study, the non-diagnostic rates were 8.8% vs. 11.0% in the two groups ($P = 0.695$), and the indeterminate rates were 1.1% vs. 9.6% ($P < 0.001$); the rate of FDD was 80.2% vs. 69.7%, ($P = 0.018$), and the accuracy was 100% vs. 94.4, ($P = 0.039$).

CONCLUSION

CEUS-guided FNAB appears to have additional benefits in FDD and accuracy compared with conventional US-guided FNAB.

CLINICAL SIGNIFICANCE

CEUS-guided FNAB can reduce repeat FNABs and diagnostic surgeries.

KEYWORDS

Bethesda, contrast-enhanced ultrasound, fine-needle aspiration biopsy, papillary thyroid carcinoma, peak intensity

Conventional ultrasound (US)-guided fine-needle aspiration biopsy (FNAB) remains the most accurate diagnostic tool for distinguishing benign from malignant thyroid nodules and is endorsed by most international guidelines.[1,2] However, FNAB has limitations, with non-diagnostic (Bethesda I) and indeterminate (Bethesda III/IV) rates each reaching up to 10%–15%, potentially leading to repeat FNAB or diagnostic hemithyroidectomy.[3] In some cases, cytological results may be inconclusive, either due to insufficient material or a lack of

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morphological diagnostic criteria. Moreover, guidelines report that the false-negative and false-positive rates of FNAB are approximately 4%–5%.[4] Methods to enhance the visibility of thyroid lesions and identify areas containing activation that improve upon conventional US merit careful consideration. Although this topic has been extensively discussed in the literature, no consensus has been reached. US elastography has been proposed to guide FNAB and reduce Bethesda I, III, or IV rates; however, the recent ElaTION randomized controlled trial found no additional benefit with this technique.[5,6]

Contrast-enhanced US (CEUS) is well established as an effective modality for assessing tissue microvascular perfusion. In recent decades, the use of CEUS for thyroid nodules has expanded considerably, owing to the development of second-generation contrast agents.[7] Heterogeneity and hypoenhancement are two major CEUS patterns associated with malignant thyroid nodules.[8,9] Contrast-enhanced US is superior to conventional US in the diagnosis of malignant thyroid nodules, with a sensitivity of 84%–85% and a specificity of 82%.[10,11] These values appear to be superior to the sensitivities and specificities reported for US alone.[12] Moreover, the false-negative rate of US-guided FNAB reportedly increases to approximately 30% in thyroid nodules with cystic components, whereas CEUS effectively identifies solid components in these mixed nodules.[13] The rationale for CEUS-guided FNAB is that CEUS accurately distinguishes viable from inactive tissues and locates viable tissues within nodules efficiently and safely. Moreover, CEUS provides precise guidance in real time for nodule puncturing.[14]

This study retrospectively compares CEUS-guided FNAB with conventional US-guided FNAB in reducing non-diagnostic and indeterminate results. In addition, it prospectively validates the added benefit of CEUS-guided FNAB for final definitive diagnosis (FDD) and accuracy.

Main points

- Contrast-enhanced ultrasound (CEUS) images are valuable in pinpointing specific areas for biopsy.
- Heterogeneity and hypo-enhancement are two major CEUS pattern characteristics of malignant thyroid nodules.
- CEUS-guided fine-needle aspiration biopsy (FNAB) could reduce repeat FNABs and diagnostic surgeries.

Methods

Study design and population

This was a pragmatic, unblinded, two-center, controlled diagnostic trial that compared the use of CEUS-guided FNAB (the trial) with conventional US-only guided FNAB (the control). Data were reviewed, and the advantages of CEUS-guided FNAB were summarized in "center 1"; however, considering the possible selection bias in the retrospective cohort, an independent, rigorous prospective cohort was used to verify the conclusions.

The study was performed in accordance with the ethical guidelines of the Helsinki Declaration. The patients were included and received full ethical approval from the ethic committee of tertiary referral center 1 and 2. Moreover, case reports and studies involving identifiable patient information, clinical images, or personal data, written informed consent were obtained from the patient.

Prospective studies had both an ethics committee approval and informed consent by participants. Retrospective studies had an ethics committee approval with waiver of informed consent.

Part 1: Retrospective evaluation of the value of contrast-enhanced ultrasound-guided fine-needle aspiration biopsy

Between April 2014 and April 2024, 5,539 thyroid nodules that underwent FNAB from center 1 were reviewed; the FNAB procedures taken by the same radiologist (who had > 10 years of expertise in thyroid FNAB) were included. A random procedure (using the parity of patients' identifier mantissa) was used to avoid chance imbalances in important stratification variables. Finally, participants were stratified at a 1:1 ratio to compare the differences between the two methods. The study was performed in accordance with the ethical guidelines of the Declaration of Helsinki. The patients were included, and the study received full ethical approval from the ethics committee of Xuzhou Center Hospital (20140144).

Part 2: Prospective evaluation of the value of contrast-enhanced ultrasound-guided in reducing indeterminate cytological diagnosis and improving diagnostic accuracy of fine-needle aspiration biopsy

This study was registered at www.chictr.org.cn (No. ChiCTR 2000035320). It is an unblinded, prospective, diagnostic trial that compares the use of CEUS-guided FNAB with conventional US-only guided FNAB. The patients were included, and the study received full ethical approval from the ethics committee Shanghai General Hospital (2024SQ241).

Between May 2024 and April 2025, thyroid nodules from consecutive patients in center 2 who underwent conventional US and FNAB by the same radiologist (with > 15 years of expertise in thyroid FNAB) were prospectively analyzed. Details of the flowchart and patient characteristics are provided in Figure 1. In the prospective cohort, patients were selected for CEUS-guided biopsy or not, as determined by the clinician; radiologists do not take part in decision-making.

Inclusion criteria were as follows: (a) aged > 18 years with nodule size > 1 cm; (b) nodules underwent conventional US and FNAB with or without CEUS.

Exclusion criteria were as follows: (a) patients with incomplete clinical or US data; (b) almost purely cystic nodules with no solid component (cystic components > 25%); (c) nodules with rim and/or eggshell calcification; (d) pregnant women.

According to the voluntary principle, patients had the right to refuse CEUS-guided FNAB in both parts of the study.

Conventional ultrasound examination

The conventional US examinations were performed using a sonography machine (Apoli 400; Toshiba, Tokyo, Japan) with a 10–14 MHz high-frequency linear transducer in center 1 and same series sonography machine (Apoli 500; Toshiba) with a 10–14 MHz high-frequency linear transducer in center 2. The greyscale US features of each thyroid nodule were recorded before FNAB. Thyroid nodules were evaluated for size [largest diameter, subgrouped as a small nodule group (< 20 mm) or a big nodule group (≥ 20 mm)], margin [well defined (0) or poorly defined (1)], internal nodule components [partially cystic (0) or solid (1)], shape [height greater than width (1) or not (0)], echogenicity [hypo-echogenicity (1), iso-echogenicity, or hyper-echogenicity relative to adjacent neck musculature (0)], and calcifications [no calcification (0) or micro-calcification (1)]. The Thyroid Imaging Reporting and Data System (TI-RADS) score (TR) was recorded: TR3 (score 1), TR4a (score 2), TR4b (score 3), TR4c (score 4) and TR5 (score 5).[15]

Contrast-enhanced ultrasound examinations before fine-needle aspiration biopsy

The CEUS acquisition and measurements were performed following the conventional US examination. A contrast agent (SonoVue, Bracco International, Milan, Italy) was intravenously injected as a bolus at a dose of 1.2–1.5 mL, followed by a 5 mL saline flush. The timer on the US machine was initiated during the CEUS process, and the images lasting ≥ 2 minutes were digitally stored as raw data.

Assessment of qualitative contrast-enhanced ultrasound

Qualitative CEUS was assessed relative to normal thyroid parenchyma. The wash-in and wash-out times of microbubbles were categorized as earlier or not; enhancement direction was classified as scattered, centripetal, or centrifugal; peak intensity was classified as hyper-, iso-, or hypo-enhancement; homogeneity of enhancement was categorized as homogeneous or heterogeneous, and rim enhancement was classified as yes or no.

Fine-needle aspiration biopsy procedures

The US-guided FNABs were performed by the experienced radiologist. At least three needle passes were required to obtain adequate specimens and followed these steps: (1) In the control group, three needle passes were well-distributed and performed in the target thyroid nodule. (2) In the CEUS-guided group, the selected solid nodule underwent a directed FNAB to the area that displayed heterogeneous hypo-enhancement in CEUS images (Figure 2). (3) If the thyroid nodule was a mix of cystic and solid regions throughout, the non-enhancing cystic or necrotic regions were avoided (Figure 3). (4) If the thyroid nodule was of uniform iso-enhancement throughout, regular FNAB was performed (Figure 4). Samples obtained with 23-gauge needles were applied to glass slides, smeared, and placed immediately in 95% ethyl alcohol for Papanicolaou staining.

Fine-needle aspiration biopsy with cytological results

The FNAB results were based on the Bethesda System for Reporting Thyroid Cytopathology, which uses a standardized, category-based reporting system for thyroid fine needle aspirations in six diagnostic categories: (I) non-diagnostic; (II) benign; (III) atypia of undetermined significance; (IV) follicular neoplasm; (V) suspicious for malignancy; and (VI) malignant (3).

Management of patients

According to the FNAB results and patient's choice, various management outcomes were implemented. Among them, definite pathological results after thyroidectomy or diagnostic surgeries were defined as primary study endpoints. For each patient, two benign FNAB results or scores of TR3 or TR4a at US with a benign FNAB result and follow-up were defined as secondary study endpoints.

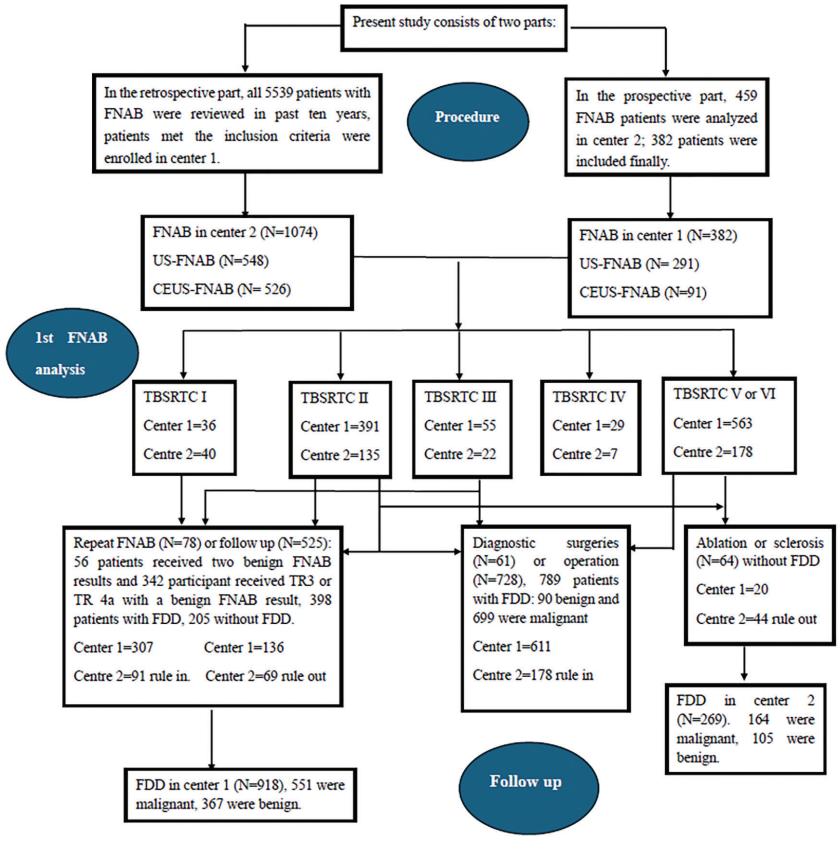

Figure 1. Flowchart of patient selection in the two centers. FNAB, fine-needle aspiration biopsy; CEUS, contrast-enhanced ultrasound; US, ultrasound; FDD, final definitive diagnosis.

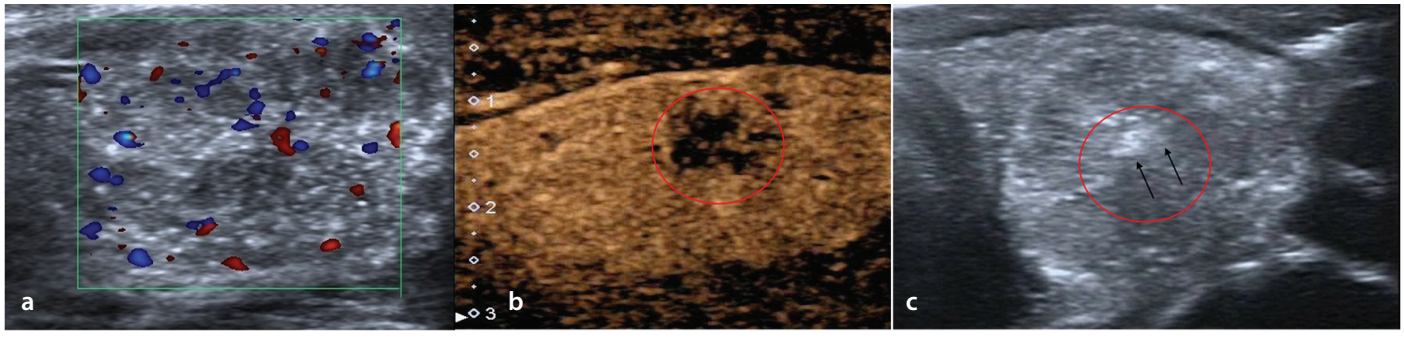

Figure 2. Images in a 24-year-old female patient with a solitary right-sided thyroid nodule classified with conventional ultrasound (US) as category TR4c and classified as Bethesda VI following cytopathologic examination. Surgical histopathologic examination confirmed papillary thyroid carcinoma. **(a)** Longitudinal view of the right thyroid lobe displaying a hypoechoic, poorly-defined nodule with microcalcifications. **(b)** Longitudinal contrast-enhanced US image showing the nodule is predominantly iso-enhanced with a hypo-enhanced area (red circle). **(c)** Transverse view of the needle within the hypo-enhancement area (red circle) in the nodule selected for cytology sampling (black arrows).

Comparison of the final definite diagnosis rate and diagnostic accuracy of fine-needle aspiration biopsy with or without contrast-enhanced ultrasound

The FNAB result was described using the Bethesda system, and patient management was analyzed. Final definitive diagnosis was defined as follows:[4,5] benign FDD – patients (i) received two benign (Bethesda II) FNAB results, (ii) received a TR3/TR4a result with US with a benign (Bethesda II) FNAB result, or (iii) underwent surgery and the nodule was found to be benign; malignant FDD–the patients received a malignant diagnosis after surgery. Moreover, in patient management, Bethesda I, III, IV, and II with TR4b or above with US without pathological results were not considered an FDD. Patients who underwent ablation or sclerosis did not reach an FDD. Accuracy was calculated by comparing the first FNAB result and the FDD.

Statistical analysis

All statistical analyses were conducted using commercially available software (Stata, version 10.0; Stata Corp, College Station, TX, US). A *P* value of <0.05 was considered indicative of statistical significance for all tests. The chi-square test was used to compare the categorical variables of clinical characteristics.

Results

Patient characteristics

In center 1, 1,074 patients with nodules met the inclusion criteria and were enrolled in the study. Finally, 548 nodules underwent conventional US-FNAB and 526 nodules underwent CEUS-FNAB. In center 2, 382 patients with nodules were included; 291 nodules underwent conventional US-FNAB and 91 nodules underwent CEUS-FNAB. There was no evidence of a difference in gender, age, nodule size, location, or complication between the two groups in both center 1 and center 2. However, in patient management, CEUS-guided FNAB resulted in fewer repeat FNABs—4.4% vs. 12.0% [risk difference: 0.365 (95% confidence interval (CI): 0.133, 1.001); $P = 0.045$] in center 2, 2.3% vs. 4.9% [risk difference: 0.463 (95% CI: 0.237, 0.904); $P = 0.022$] in center 1—and fewer diagnostic surgeries—0% vs. 5.8% [risk difference: 0.091 (95% CI: 0.006, 1.493); $P = 0.016$] in center 2, 3.4% vs. 4.7% [risk difference: 0.721 (95% CI: 0.400, 1.300); $P = 0.286$] in center 1 (Table 1).

Conventional US features and TI-RADS assessment before FNAB are presented in Table 2. Of the 1,456 nodules, 64 (4.4%) were assessed as benign (TR3 category), 360 (27.2%) as slightly suspicious (TR4a category), 499 (34.3%) as moderately suspicious (TR4b category), 402 (27.6%) as severely suspicious (TR4c category), and 95 (6.5%) as malignant (TR5 category). There was no significant difference in TR assessment between center 1 and center 2. Of the 1,456 patients, 1,187 (81.5%) reached an FDD (Table 2).

Ninety-one patients from center 2 and 526 patients from center 1 underwent CEUS before FNAB. The CEUS characteristics of 564 thyroid nodules with FDD from the two centers are presented in Table 3. Homogeneity and peak intensity showed significant differences between malignant groups and benign groups: 304 of 356 (85.4%) in the malignant group vs. 164 of 208 (78.8%) in the benign group displayed hypo-enhancement ($P < 0.001$). More nodules in the malignant group had heterogeneous enhancement (80.6%, 287/356) at CEUS than in the benign group (38.5%, 80/208) ($P < 0.001$). There were no statistically significant differences in other CEUS characteristics between the malignant and benign groups. The sensitivity, specifici-

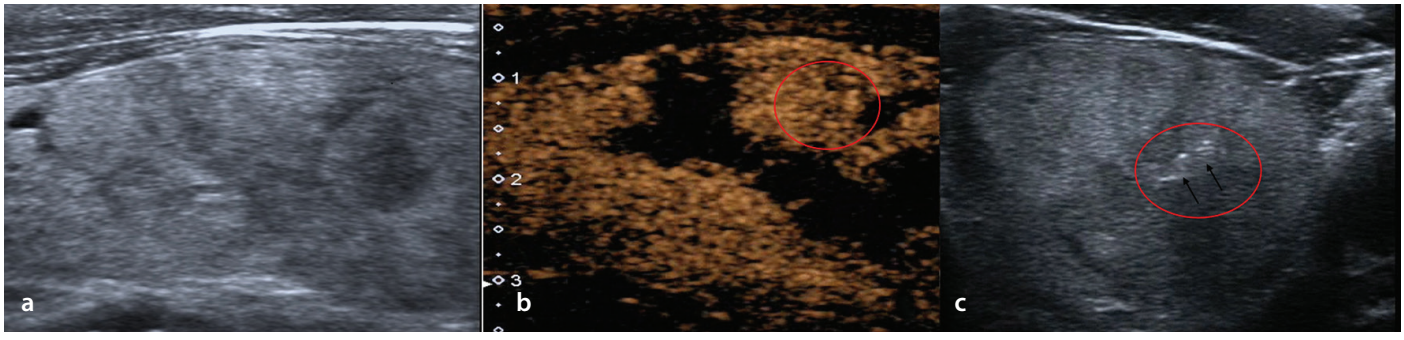

Figure 3. Images in a 39-year-old male patient with a solitary left-sided thyroid nodule classified with conventional ultrasound (US) as category TR4a and classified as Bethesda II following cytopathologic examination. Repeat US in the following 7 years showed no further change and no further intervention was necessary. **(a)** Longitudinal view of the left thyroid lobe displays an iso-echo, well-defined solid nodule. **(b)** Longitudinal contrast-enhanced US image shows the nodule is predominantly iso-enhanced with cystic areas, which were invisible with conventional US (red circle). **(c)** Transverse views of the needle area without cystic change were selected for cytology sampling (black arrows).

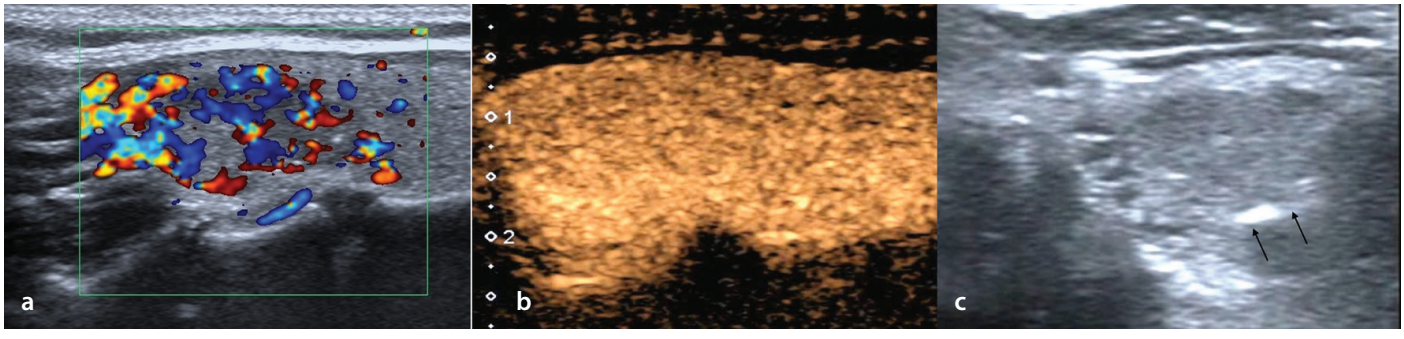

Figure 4. Images in a 64-year-old female patient with a solitary right-sided thyroid nodule classified with conventional ultrasound (US) as category TR4a and classified as Bethesda II following cytopathologic examination. Repeat fine-needle aspiration biopsy (FNAB) was performed 2 years later, showing Bethesda II again. **(a)** Longitudinal view of the right thyroid lobe displays a hypo-echogenic, well-defined solid nodule. **(b)** Longitudinal contrast-enhanced US image shows the nodule has homogeneous iso-enhancement. **(c)** Transverse views show where the regular FNAB was performed (black arrows).

ty, positive predictive value, negative predictive value, and accuracy for heterogeneous enhancement to predict malignancy were 80.6%, 61.5%, 78.2%, 64.9%, and 73.5%, respectively; for hypo-enhancement, the values were 85.3%, 21.2%, 64.9%, 45.8%, and 61.7%, respectively.

In center 1, the non-diagnostic (Bethesda I) rate following the first FNAB in the CEUS group was lower than in the US group: 2 of 526 (0.4%) vs. 34 of 548 (6.2%) [risk difference: 0.061 (95% CI: 0.015, 0.254); $P = 0.000$]. The indeterminate diagnostic rate (Bethesda III or IV) was higher in the CEUS-guided group [23 of 526 (4.4%)] and the US-guided group [61 of 548 (11.1%)] [risk difference: 0.393 (95% CI: 0.247, 0.624); $P = 0.000$].

In center 2, the non-diagnostic rate was 8 of 91 (8.8%) for CEUS-FNAB vs. 32 of 291 (11.0%) for US-FNAB [risk difference: 0.799 (95% CI: 0.382, 1.672); $P = 0.695$]. The indeterminate diagnostic rate was 1 of 91 (1.1%) in the CEUS group vs. 28 of 291 (9.6%) in the US-guided group [risk difference: 0.114 (95% CI: 0.016, 0.828); $P = 0.032$].

Final definitive diagnosis results

In center 1, 491 of 526 (93.3%) patients with CEUS-guided FNAB had an FDD: 192 had a benign FDD, and 299 had a malignant FDD; 427 of 548 (77.9%) patients with US-guided FNAB had an FDD: 175 had a benign FDD and 252 had a malignant FDD [risk difference: 1.198 (95% CI: 1.139, 1.259); $P = 0.000$].

In center 2, 73 of 91 (80.2%) patients with CEUS-guided FNAB had an FDD: 16 had a benign FDD and 57 had a malignant FDD; 196 of 291 (67.3%) patients with US-guided FNAB

Table 1. Clinical characteristics of participants according to intervention group from two tertiary referral centers

Basic characteristics	The retrospective study from center 1		Total	*P* value	The prospective study from center 2		Total	*P* value
	CEUS-guided FNAB	US-guided FNAB	1,074		CEUS-guided FNAB	US-guided FNAB	382	
Gender	526	548		0.206	91	291		0.301
Male	168	155	323		33	87	120	
Female	358	393	751		58	204	262	
Mean age, y	49.5 ± 15.1	51.5 ± 14.9		0.260	51.4 ± 14.9	53.6 ± 14.5		0.222
Mean nodule, mm	15.9 ± 7.7	15.5 ± 7.0		0.298	13.5 ± 6.2	13.5 ± 7.1		0.170
Location				0.326				0.154
Right lobe	242	229	471		42	167	209	
Left lobe	263	299	562		46	114	160	
Isthmus	21	20	41		3	10	13	
Composition				0.481				0.176
Solid	503	518	1,021		85	256	342	
Mixed cystic and solid	23	30	53		6	35	40	
Echogenicity				0.183				0.181
Hypo-echogenic	496	512	1,008		85	272	357	
Iso-echogenic	29	30	53		4	18	22	
Hyper-echogenic	1	6	13		2	1	3	
Shape				0.193				0.416
Wider-than-tall	396	431	827		74	247	321	
Taller-than-wide	130	117	247		17	44	61	
Margin				0.459				0.116
Well defined	296	321	617		58	211		
Poorly defined	230	227	457		33	80		
Calcification				0.137				0.280
Microcalcification	319	307	608		50	139	189	
No calcification	207	241	466		41	152	193	
TI-RADS				0.052				0.054
TR3	10	7	17		8	39	47	
TR4a	132	170	302		15	79	94	
TR4b	164	186	350		39	110	149	
TR4c	180	149	329		25	48	73	
TR5	40	36	76		4	15	19	

FNAB, fine-needle aspiration biopsy; CEUS, contrast-enhanced ultrasound; US, ultrasound; TI-RADS, Thyroid Imaging Reporting and Data System.

Table 2. FNAB results and management of patients according to intervention group

Basic characteristics	The retrospective study from center 1		Total	*P* value	The prospective study from center 2		Total	*P* value
	CEUS-guided FNAB	US-guided FNAB	1,074		CEUS-guided FNAB	US-guided FNAB	382	
Bethesda category	526	548	1,074	0.000	91	291	382	0.004
I	2	34	36		8	32	40	
II	195	196	391		24	111	135	
III	9	46	55		1	21	22	
IV	14	15	29		0	7	7	
V	79	14	93		4	8	12	
VI	227	243	470		54	112	166	
Complication				0.155				0.563
Hematoma	15	25	40		3	5	8	
Swelling and etc.	8	14	22		0	1	1	
None	503	509	1,012		88	285	373	
Management				0.000				0.000
Follow up	175	229	404		21	100	121	
Repeat FNAB	12	27	39		4	35	39	
Ablation or sclerosis	13	7	20		9	35	44	
Diagnostic surgeries	18	26	44		0	17	17	
Operation	308	259	567		57	104	161	
FDD	491	427	918	0.000	73	196	269	0.000
Histological result	326	285	611		57	133	190	
Two Bethesda II	10	7	17		0	8	8	
Bethesda II and TR3 or 4a	155	135	290		16	55	71	
Accuracy	490/491	419/427		0.015	73/73	185/196		0.039

FNAB, fine-needle aspiration biopsy; CEUS, contrast-enhanced ultrasound; US, ultrasound; FDD, final definitive diagnosis; TR, Thyroid Imaging Reporting and Data System category.

Table 3. Contrast-enhanced ultrasound characteristics of 564 thyroid nodules with FDD in two centers

CEUS feature	Benign group (n = 208)	Malignant group (n = 356)	FDD (n = 564)	*P* value
Earlier wash-in				1.000
Yes	2	3	5	
No	206	353	559	
Enhancement direction				0.188
Scattered	58	81	139	
Centripetal or centrifugal	150	275	425	
Homogeneity				0.000
Homogeneous	128	69	197	
Heterogeneous	80	287	367	
Peak intensity				0.000
Hypo-enhancement	164	304	468	
Iso-enhancement	40	27	67	
Hyper-enhancement	2	22	24	
Without-enhancement	2	3	5	
Rim enhancement				0.103
Yes	8	26		
No	200	330		
Earlier wash-out				1.000
Yes	2	3	5	
No	206	353	559	

had an FDD: 89 had a benign FDD and 107 had a malignant FDD [risk difference: 1.191 (95% CI: 1.046, 1.356); $P = 0.008$].

There were statistically significant differences in accuracy between the two groups, both in center 1 [99.8% (490/491) vs. 98.1% (419/427); risk difference: 1.017 (95% CI: 1.003, 1.031); $P = 0.015$] and in center 2 [100.0% (73/73) vs. 94.4% (185/196); risk difference: 1.039 (95% CI: 1.003, 1.076); $P = 0.015$].

Discussion

As far as we know, few prospective studies evaluating CEUS-guided FNAB of thyroid nodules have been reported. This trial in thyroid nodules has shown that FNAB appears to benefit from preoperative CEUS guidance. Comparing our findings with previously published studies evaluating US elastography-guided FNAB, CEUS reduced the non-diagnostic rate (Bethesda I) and the indeterminate diagnostic rate (Bethesda III or IV) of FNAB in the retrospective cohort, which caused a significant reduction in the number of repeated FNAB or unnecessary thyroidectomies in the prospective cohort, whereas US elastography does not appear to have additional benefit over conventional US. The main reason may be that CEUS could display the heterogeneity in thyroid nodules more effectively than US elastography. It is worth noting that the non-diagnostic rate under CEUS guidance was not significantly different from under US guidance (8.8% vs. 11.0%, $P = 0.695$). This discrepancy was attributed to the small sample size, lower proportion of CEUS-guided FNAB, and more mixed cystic and solid thyroid nodules in the prospective cohort. A high rate of inadequate thyroid epithelium on FNAB exists when thyroid nodules have a cystic component.[16,17] Therefore, it remains to be demonstrated whether the conclusions of retrospective studies are valid in prospective studies.

As well as avoiding unnecessary FNABs in cystic areas, a previous review[18] reported that hypoenhancement is the key CEUS feature of malignant thyroid nodules. The present trial confirms this classical conclusion: the radiologist found that a total of 65.0% of CEUS applications were useful in identifying which area of the thyroid nodule to biopsy. Therefore, we also undertook an assessment of the CEUS results by the senior operator who may have the most intuitive feeling before FNAB. Moreover, CEUS could display the solid component in mixed cystic–solid thyroid nodules to improve the specimen adequacy of FNAB.

This study has several strengths: although CEUS is a diagnostic technique that has been widely used for thyroids in the past decade, few centers had experience in guiding thyroid FNAB. Our study implemented a 10-year certified program consisting of two referral centers. The results showed that FDD and accuracy demonstrated significant differences in both the retrospective and prospective parts. Moreover, the same serial sonography machines were used in the two centers, which would further minimize systematic or test errors, demonstrating that the quality of CEUS reporting was high and consistent.

Our study has several limitations. First, the participants in center 1 were retrospectively reviewed, and the research period spans 10 years. The Bethesda category was analyzed by several cytologists, which may potentially lead to observer bias. Second, in the prospective cohort, patients were selected for CEUS-guided biopsy according to the clinician's decision and the patient's voluntary choice rather than random allocation; the proportion of CEUS-guided and conventional US-guided biopsies was 1:3, which may have selection bias. Third, there is also a finding that CEUS results vary by pathology type; CEUS is best for papillary thyroid carcinoma, which displays homogeneous and heterogeneous enhancement, whereas follicular and medullary thyroid cancers display hyperenhancement; therefore, CEUS-guided FNAB is unlikely to be useful in these cases. Fourth, the definition of FDD includes the FNAB result itself, and histopathological confirmation was available only in selected patients. This may introduce incorporation bias and potentially verification bias, which partly explains the high diagnostic accuracy values. Moreover, the largest clinical application of CEUS is with TI-RADS category IV before FNAB in the present study; not all thyroid nodules were expected to benefit. Finally, there was a low malignancy rate for nodules graded non-diagnostic (Bethesda I) at second FNAB that went on to have an FDD. Furthermore, most Bethesda I nodules were discharged without a final diagnosis because they were considered very low risk after CEUS assessment. In addition, the benignity rate (54/113) of the indeterminate subgroup (Bethesda III and IV) was higher.

In conclusion, the CEUS guidance approach can enhance the efficacy of FNAB. The CEUS method presents significant potential for improving the FDD of FNAB.

Footnotes

Conflict of interest disclosure

The authors declared no conflicts of interest.

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