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*Original Article*

# Comparison of contrast-enhanced ultrasound and conventional ultrasound for guiding peripheral pulmonary biopsies

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**Abstract:** Objective: To compare the clinical value of CEUS vs. conventional ultrasound for guiding peripheral pulmonary biopsies of space-occupying lesions. Methods: This was a retrospective study of the patients that underwent a US- or CEUS-guided lung biopsy between January 2013 and August 2015 at the Zhongshan Hospital affiliated to Fudan University. CEUS examination was performed using the SonoVue contrast agent. Patients underwent CEUS- or US-guided lung biopsy. Results: Patient characteristics were similar between the two groups, but lesions were larger (P=0.017) in the CEUS group. The success rate of CEUS-guided biopsies was better than that of US-guided biopsies (97.5% vs. 86.3%, P=0.002). When distinguishing malignant from benign lesions, for CEUS, sensitivity was 95.1%, specificity was 100.0%, positive predictive value was 100.0%, and negative predictive value was 90.9%, for an overall accuracy of 96.7%, compared with 94.7%, 100.0%, 100.0%, 93.2%, and 97.0% for US. When considering the individual diagnoses, consistency between biopsy and surgery was 95.9% for CEUS-guided lung biopsy, compared with 87.0% for US-guided lung biopsy (P=0.013). In the US group, there were three cases of hemoptysis, one of pneumothorax, and one of chest pain. In the CEUS group, there were one case of hemoptysis and one of chest pain. Conclusion: CEUS-guided biopsy of peripheral pulmonary lesions could have a better diagnostic accuracy than that of US-guided biopsy, and could be associated with less complication.

**Keywords:** Ultrasound, contrast-enhanced ultrasound (CEUS), pulmonary lesions, biopsy

## Introduction

Transthoracic lung biopsy is regarded as an important tool for the diagnosis of lung cancer [1, 2]. Ultrasound (US)-guided biopsy of lung lesions is an effective method that yields enough tissue for histopathological diagnosis. Compared with conventional computed-tomography (CT)-guided biopsies, US has advantages such as easy implementation and operation, low price, no radiation, real-time imaging, synchronization with respiration, and repeatable operation, and has a comparable diagnostic accuracy [3-5]. Compared with conventional bronchoscopy, US has advantages such as better tissue harvest, less trauma, and low requirement from the patient [6]. Nevertheless, false negatives on US still represent 12% of the cases, mainly due to necrosis in lung tumors [5, 7-10]. Finding new ways to improve the diagnostic performances of US is of importance.

In recent years, with the development of contrast-enhanced ultrasound techniques, contrast-enhanced ultrasound (CEUS)-guided lung biopsies can now clearly show the blood supply and necrosis area of the tumor [11-13]. When guiding the biopsy, real-time monitoring is performed to avoid the large blood vessels and necrosis area. Compared with conventional US-guided biopsy, CEUS significantly increases the satisfaction for tissue harvest and the success rate for puncture [10], but data are still lacking for comparing the two approaches for lung lesions.

Therefore, the aim of the present study was to compare the clinical value of CEUS vs. conven-

tional US for guiding peripheral pulmonary biopsies of space-occupying lesions.

## Materials and methods

### *Study design and patients*

This was a retrospective study of the patients that underwent a US- or CEUS-guided peripheral pulmonary biopsy between January 2013 and August 2015 at the Zhongshan Hospital affiliated to Fudan University. The ethical committee of the Zhongshan Hospital approved this study. The need for individual consent was waived by the committee because of the retrospective nature of the study.

Inclusion criteria were: 1) presence of a peripheral pulmonary space-occupying lesion by CT, confirmed by US; and 2) peripheral pulmonary biopsy was performed under US or CEUS guidance. Exclusion criteria were: 1) severe underlying heart or lung diseases such as myocardial infarction or chronic obstructive pulmonary disease; 2) patients with poor general condition that could not tolerate puncture or were unable to cooperate for completing the biopsy; 3) medical history of allergic reaction to iodinated contrast media; or 4) bleeding tendency with a clotting time >17 seconds or platelet count <40,000/ml.

### *Imaging*

All patients underwent CT examination to reveal the lung space-occupying lesions near the chest wall. Based on CT images, B-mode US examination was performed (Philips HD15 UI, Best, The Netherland) with a C5-2 probe at 2-5 MHz. Imaging was first done with the patients were supine in the lateral or prone position, followed by upper limb elevation to allow full spread of the intercostal space. US was used to carefully scan the lung on the diseased side. When the lesions were found, the size, shape, location, and blood supply of the lesions, important and large blood vessels around the tumor tissue, and the presence of necrosis in the tumor were recorded.

### *Contrast-enhanced ultrasound*

CEUS examination was performed using the SonoVue contrast agent (Bracco, Mila, Italy) prepared with 25 mg of dry powder, 59 mg of SF6 gas and 5 ml of normal injectable saline. All ingredients were placed in the bottle and fully shaken to form microbubbles suspension. Then, 2.4 ml of contrast agent was injected into a forearm superficial vein, and 5 ml of saline was injected for quickly washing. The US system was set at a mechanical index of 0.1 and was operated by a deputy chief radiologist with a senior attending physician (>10 years of experience). In case of discrepancy, the deputy chief physician was consulted. Enhanced beginning time, peak time, echo time, decreased time (surrounding liver tissue, spleen, or pleural tissue was considered as control [14]), enhanced way, availability of large blood vessels around, and presence/absence of internal necrosis were observed, followed by recording with static and dynamic instrument for at least 3 min.

### *Ultrasound-guided biopsy*

After CEUS or CT alone (for those who underwent US-guided biopsy), the best position and puncture pathway were selected. Tumor necrosis area was avoided and an area of enhanced activity was selected for puncture. Local disinfection and local anesthesia with 2% lidocaine were performed first, followed by puncture using a 16G puncture needle and puncture gun (Bard Biopsy Systems, Tempe, AZ, USA) under US guidance. The puncture needle was guided into an active region of the tumor. Patients were required to hold their breath during the biopsy. The harvested tissues were visually observed to evaluate breaking and necrosis. As per routine procedures, two punctures were performed, if possible. If the tissue was broken or if the amount was too small, an additional puncture was performed. Tissues was immediately placed in 5% formaldehyde for fixation and sent for pathological examination. After the puncture was completed, the patient was kept lying down and observed for 1-2 h to observe for any hemoptysis, shortness of breath, chest pain, palpitation, and other complications.

### Pathological examination

Biopsy samples were routinely processed and examined by experiences pathologists. Any patients with a benign result but with imaging features of aggressiveness were followed up by imaging or had a second-intention biopsy, either transthoracic or by bronchoscopy.

The biopsy was considered as a true positive when the pathological examination of the surgi-

**Table 1.** Characteristics of the patients and biopsies

| | CEUS (n=121) | US (n=131) | P |
|---|---|---|---|
| Age, median (range) | 62 (20, 91) | 61 (16, 86) | 0.804 |
| Age, mean ± SD | 60.2±14.8 | 59.6±15.1 | |
| Gender | | | 0.435 |
| Male | 85 (70.2%) | 86 (65.6%) | |
| Female | 36 (29.8%) | 45 (34.4%) | |
| Pathologic result of puncture | | | 0.308 |
| Benign | 44 (36.4%) | 59 (45.0%) | |
| Malignant | 77 (63.6%) | 72 (55.0%) | |
| Lesion size ($mm^2$) | 2275 (288, 14946) | 1716 (500, 8715) | 0.017 |
| | 3105.94±2711.04 | 2212.36±1560.36 | |
| Position | | | 0.206 |
| Right side | 66 (54.5%) | 61 (46.6%) | |
| Left side | 55 (45.5%) | 70 (53.4%) | |
| Adverse reaction | 2 (1.7%) | 5 (3.8%) | 0.449 |
| Number of punctures | 2 (2, 3) | 2 (2, 3) | 0.853 |
| | 2.08±0.27 | 2.08±0.27 | |
| 2 | 111 (91.7%) | 121 (92.4%) | 0.853 |
| 3 | 10 (8.3%) | 10 (7.6%) | |
| Success rate of puncture | | | 0.002 |
| Success | 118 (97.5%) | 114 (87.0%) | |
| Failure | 3 (2.5%) | 17 (13.0%) | |

cal specimen and the biopsy both revealed lung cancer. False negative was considered when the biopsy was benign but subsequent examinations revealed cancer.

*Evaluation indexes*

The success rate of the biopsy, puncture time, adverse reactions, malignant detection rate, diagnostic indexes were evaluated between the US and CEUS groups. Indicator of contrast ultrasound between benign and malignant tissues was evaluated. The average enhancing time and peak time were evaluated between benign and malignant tissues. Beginning of lesion enhancement, mean time to enhancement, and time to peak enhancement of lesions were divided into fast-forward (<40 s), fast-out (between 40 and 120 s), fast-forward and slow-out (>120 s), fast-forward but no out, slow-forward and fast-out, slow-forward and slow-out, slow-forward but no out.

*Statistical analysis*

Categorical data are presented as frequencies and percentages and were analyzed using the pearson chi-square or Fisher's exact test. For continuous variables, normality of the distribution was tested using the Smirnov-Kolmogorov test. Normally-distributed data are presented as mean ± standard deviation and were tested using the Student's t test. Non-normally distributed data are presented as median (range) and were analyzed using the Mann-Whitney U test. Diagnostic sensitivity, specificity, positive predictive value, negative predictive value, accuracy for distinguish malignant and benign lesions were calculated. All analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Two-sided *P*-values <0.05 were considered statistically significant.

## Results

*Characteristics of the patients and biopsies*

**Table 1** presents the characteristics of the patients and biopsies, 121 patients underwent CEUS-guided biopsy while 131 underwent US-guided biopsy. Age, gender, biopsy result, position, adverse reactions, and number of punctures were similar between the two groups, but lesions were larger (P=0.017) in the CEUS group. In addition, the success rate of CEUS-guided biopsies was better than that of US-guided biopsies (97.5% vs. 86.3%, P=0.002). **Table 2** presents the descriptive statistics of the CEUS examinations.

*Pathological results*

**Table 3** presents the pathological results. There was no difference in the frequencies of the different diagnoses between the two groups.

*Diagnostic accuracy*

**Table 4** presents the diagnostic accuracy of CEUS-guided lung biopsy according to the

**Table 2.** Characteristics of the CEUS examinations

| Parameters | Descriptive statistics |
|---|---|
| Internal echo | |
| Yes | 25 (20.8%) |
| No | 95 (7.2%) |
| Uniformity | |
| Uniform | 7 (5.8%) |
| Less uniform | 77 (64.2%) |
| Non-uniform | 36 (30.0%) |
| Pattern | |
| Regular | 12 (10.2%) |
| Less regular | 55 (46.6%) |
| Irregular | 51 (43.2%) |
| Delay period | |
| Equal echo | 21 (17.4%) |
| Low echo | 100 (82.6%) |
| Enhanced area | |
| Yes | 78 (64.5%) |
| No | 43 (35.5%) |
| Angiographic features | |
| Fast-forward but no out | 12 (10.08%) |
| Fast-forward and fast-out | 77 (64.71%) |
| Fast-forward and slow-out | 29 (24.37%) |
| Slow-forward and fast-out | 1 (0.84%) |
| CEA, mean ± SD | 10.79±21.51 |
| CEA, median (range) | 2.7 (0.6, 87.6) |
| Cytokeratin-19, mean ± SD | 9.58±13.54 |
| Cytokeratin-19, median (range) | 4.2 (0.5, 52.9) |
| Neuron-specific enolase, mean ± SD | 29.39±52.45 |
| Neuron-specific enolase, median (range) | 15.75 (8.5, 260.4) |
| Squamous cell carcinoma antigen, mean ± SD | 1.94±2.66 |
| Squamous cell carcinoma antigen, median (range) | 0.7 (0.4, 11.5) |
| Resistance index, mean ± SD | 0.62±0.13 |
| Resistance index, median (range) | 0.61 (0.3, 1) |
| Beginning time, mean ± SD | 13.52±4.44 |
| Beginning time, median (range) | 13 (6, 27) |
| Peak time, mean ± SD | 23.8±6.81 |
| Peak time, median (range) | 23 (11, 50) |
| Fade time, mean ± SD | 45.13±18.53 |
| Fate time, median (range) | 40 (20, 100) |

examination of the surgical specimen as the gold standard. When distinguishing malignant from benign lesions, sensitivity was 95.1%, specificity was 100.0%, positive predictive value (PPV) was 100.0%, and negative predictive value (NPV) was 90.9%, for an overall accuracy of 96.7%.

**Table 5** presents the diagnostic accuracy of US-guided lung biopsy according to the examination of the surgical specimen as the gold standard. When distinguishing malignant from benign lesions, sensitivity was 94.7%, specificity was 100.0%, PPV was 100.0%, and NPV was 93.2%, for an overall accuracy of 97.0%.

**Table 6** presents the diagnostic accuracy of the two biopsy approaches according to the specific diagnoses. When considering the individual diagnoses, consistency between biopsy and surgery was 95.9% for CEUS-guided lung biopsy, compared with 87.0% for US-guided lung biopsy (P=0.013).

*Enhancement patterns*

Among benign lesions, 28.2% of the lesions showed a fast-forward but no out pattern, 33.3% showed fast-forward and slow-out, and 38.5% showed fast-forward and fast-out, compared with 1.3%, 20.0%, and 78.8%, respectively, for malignant lesions (P<0.001).

*Adverse reactions*

In the US-guided biopsy group, there were three cases of hemoptysis, one case of pneumothorax, and one case of chest pain. In the CEUS-guided biopsy group, there were one case of hemoptysis and one case of chest pain. All adverse reactions were relieved after symptomatic treatment.

*Typical cases*

The Supplementary Materials present some typical cases: lung adenocarcinoma (Supplementary Figure 1), lung inflammation (Supplementary Figure 2), lung granuloma (Supplementary Figure 3), small cell carcinoma of the lung (Supplementary Figure 4), and squamous cell lung carcinoma (Supplementary Figure 5).

**Table 3.** Final diagnosis according to the type of biopsy

| | CEUS (n=121) | US (n=131) | P |
|---|---|---|---|
| Squamous Cell Carcinoma | 23 (19.0%) | 23 (18.5%) | 0.766 |
| Adenocarcinoma | 29 (24.00%) | 36 (29.0%) | 0.524 |
| Small cell carcinoma | 9 (7.4%) | 6 (4.8%) | 0.338 |
| Metastatic lung cancer | 6 (5.0%) | 5 (4.0%) | 0.658 |
| Other malignancies | 14 (11.6%) | 6 (4.8%) | 0.040 |
| Tuberculous granulomas with necrosis | 14 (11.6%) | 12 (9.7%) | 0.530 |
| Lung abscess or inflammation | 24 (19.8%) | 36 (29.0%) | 0.155 |
| Other benign tumors | 2 (1.7%) | 7 (5.3%) | 0.288 |
| Total | 81 (66.9%) | 76 (58.0%) | 0.144 |

**Table 4.** Diagnostic accuracy of CEUS-guided peripheral pulmonary biopsy

| Enhanced ultrasound | | Final diagnosis | | Total |
|---|---|---|---|---|
| | | Malignant | Benign | |
| Puncture pathology | Malignant | 77 (95.1%) | 0 | 77 (63.6%) |
| | Benign | 4 (4.9%) | 40 (100.0%) | 44 (36.4%) |
| Total | | 81 (100.0%) | 40 (100.0%) | 121 (100.0%) |
| Sensitivity | | 95.1% | | |
| Specificity | | 100.0% | | |
| Positive predictive value | | 100.0% | | |
| Negative predictive value | | 90.9% | | |
| Accuracy | | 96.7% | | |

**Table 5.** Diagnostic accuracy of the US-guided peripheral pulmonary biopsy

| Normal ultrasound | | Final diagnosis | | Total |
|---|---|---|---|---|
| | | Malignant | Benign | |
| Puncture pathology | Malignant | 72 (94.7%) | 0 | 72 (55.0%) |
| | Benign | 4 (5.3%) | 55 (100.0%) | 59 (45.0%) |
| Total | | 76 (100.0%) | 55 (100.0%) | 131 (100.0%) |
| Sensitivity | | 94.7% | | |
| Specificity | | 100.0% | | |
| Positive predictive value | | 100.0% | | |
| Negative predictive value | | 93.2% | | |
| Accuracy | | 97.0% | | |

## Discussion

Contrast-enhanced ultrasound (CEUS)-guided biopsies have advantages compared with computed tomography-guided biopsies, but whether it is better than conventional ultrasound is still poorly known. Therefore, the present study aimed to compare the clinical value of CEUS vs. conventional ultrasound for guiding lung biopsies of space-occupying lesions. Results showed that the success rate of CEUS-guided biopsies was better than that of US-guided biopsies. When considering malignant vs. benign diagnoses, the two approaches had similar diagnostic accuracy, but when considering the individual diagnoses, CEUS-guided lung biopsy was correct in 95.9% of the cases, compared with 87.0% for US-guided lung biopsy. Therefore, CEUS-guided biopsy of peripheral lung tumor could have a better diagnostic accuracy than that of US-guided biopsy.

Before the advent of CEUS, US was used to determine the best needle path [15], but this approach heavily relied on the experience of the radiologist in being able to distinguish small blood vessels with a low flow rate. SonoVue™ is a second-generation US contrast agent that has gas-filled microbubbles (around 7 μm) that are smaller than erythrocytes and that can travel freely in the circulatory system, including capillaries [16, 17]. Previous preliminary studies revealed that CEUS could be used to improve the selection of the needle path for lung biopsy [10, 18] and that CEUS was useful to differentiate lung tumors from benign pathological processes [15, 19]. In the present study, biopsy success (i.e. for obtaining enough tissues for analysis) was 97.5% with CEUS-guided biopsy compared with 86.3% for US-guided biopsy, which is similar to a previous study (98.1%) [11].

Previous studies reported a diagnostic accuracy of US-guided lung biopsies ranging from 91% to 96% [20, 21]. Using US alone, some specimens cannot be examined because of necrosis

**Table 6.** Diagnostic accuracy of CEUS- vs. US-guided peripheral pulmonary biopsies according to the final diagnosis

| Diagnostic accuracy of puncture | | | | |
|---|---|---|---|---|
| Final diagnosis | N | Contrast group | Non-contrast group | P |
| Squamous cell carcinoma | 46 | 23/23 | 23/23 | |
| Adenocarcinoma | 65 | 28/29 | 34/36 | |
| Small cell carcinoma | 15 | 8/9 | 5/6 | |
| Metastatic cancer | 11 | 6/6 | 4/5 | |
| Other malignancies | 20 | 12/14 | 6/6 | |
| Granuloma | 26 | 14/14 | 12/12 | |
| Inflammation | 60 | 23/24 | 30/36 | |
| Other benign tumors | 9 | 2/2 | 0/7 | |
| Correct diagnosis | 252 | 116/121 (95.9%) | 114/131 (87.0%) | 0.013 |

[8, 10]. CEUS allows the visualization of the blood supply, hereby avoiding necrosis areas without blood supply and targeting well-vascularized areas that are more likely to be malignant [10, 11]. Studies of CEUS-guided lung biopsy revealed diagnostic accuracy ranging from 85 to 98% [10, 20, 21]. The present study showed that CEUS- and US-guided lung biopsies had similar diagnostic accuracies when considering the lesions in terms of malignant/benign, but the accuracy of CEUS-guided biopsy was better when considering individual diagnoses, which is probably due to a better targeting of areas of interest, as shown by previous studies [10, 11]. A study reported an accuracy of 96% for CEUS-guided biopsy compared with 80% for US [22]. The lack of difference in the present study between CEUS and US could be due to a number of reasons, the main one probably being the experience of the radiologists in distinguishing small vessels on conventional US.

Enhancement patterns of lung lesions can be divided into fast-forward and fast-out, fast-forward and slow-out, and fast-forward but no out. Liver, spleen, and kidney are generally considered as control, but sometimes pleura or surrounding soft tissues can also be used as reference [10, 11]. The fast-forward and fast-out pattern is usually the dominant pattern in malignant tumors [10-14, 16-19]. The blood vessels are distorted and disordered, and are often part of a lump or nodular lesion. In addition, a malignant lesion is more likely to contain necrosis foci [10, 11, 18, 22]. In the present study, most malignant lesions showed the fast-forward and fast-out pattern, which is supported by the studies above.

Lung biopsies are associated with possible adverse reactions such as hemoptysis and pneumothorax [11]. In the present study, these events were rare. CEUS-guided biopsy seemed to be associated with fewer adverse reactions than US-guided biopsy, as previously observed [10, 11], but the low rate of events prevented reliable analyses. Nevertheless, better visualization of the blood supply could have played a role in the lower frequency of adverse events.

Of course, the present study is not without limitations. The sample size was small and from a single center. In addition, as for any ultrasound technique, CEUS is operator-dependent [23], which have could lead to fewer differences between US and CEUS probably because of the ability of the radiologists in detecting small vessels on conventional US. Additional multicenter studies are necessary to validate these results.

In conclusion, CEUS-guided biopsy of peripheral pulmonary tumor could distinguish well for necrosis and active areas, so it have a better success rate of the biopsy than that of US-guided biopsy, and could be associated with less complication. Additional studies are still necessary to determine the benefits of CEUS over US.

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## Disclosure of conflict of interest

None.

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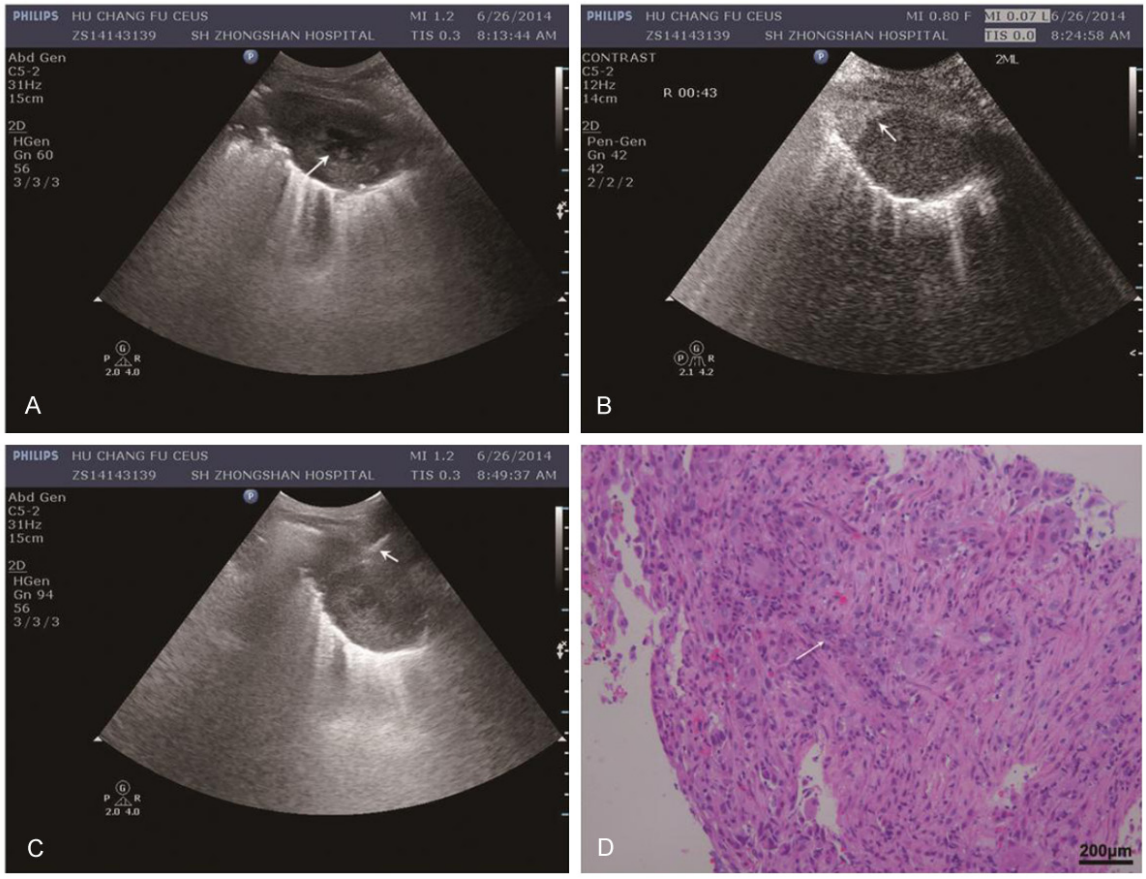
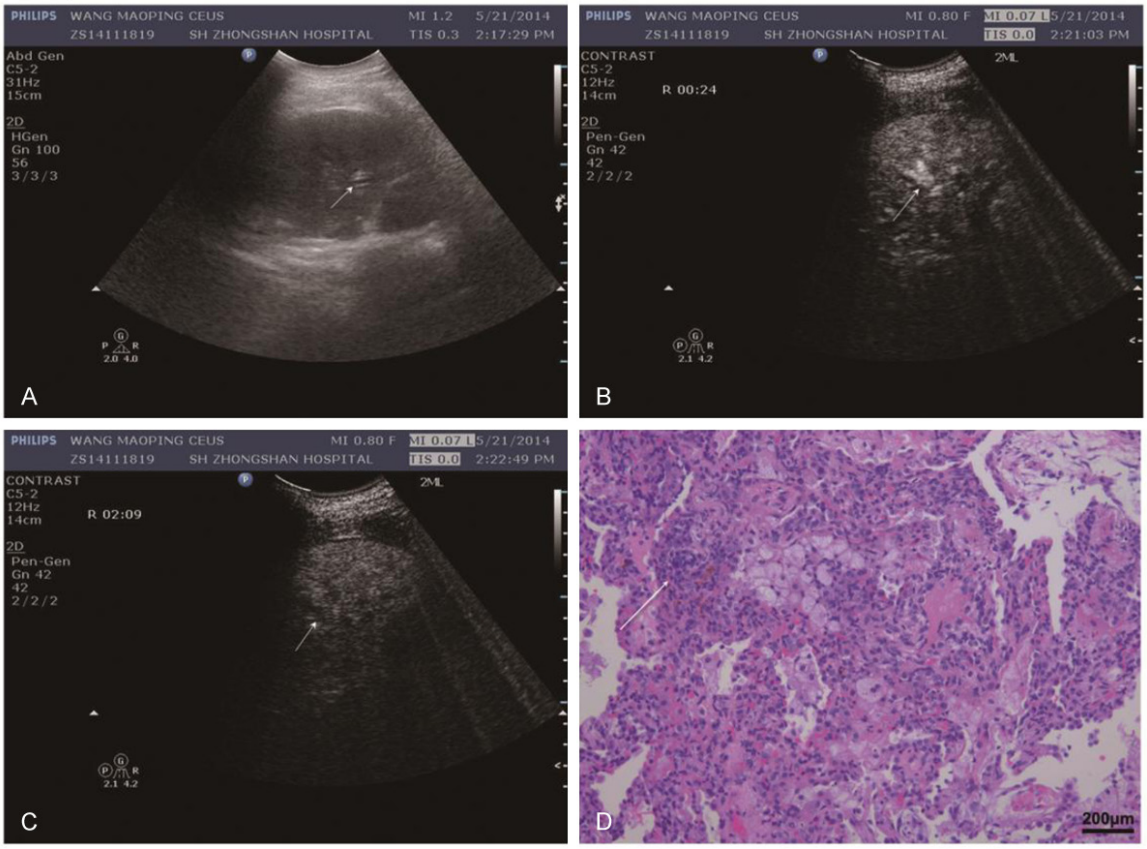
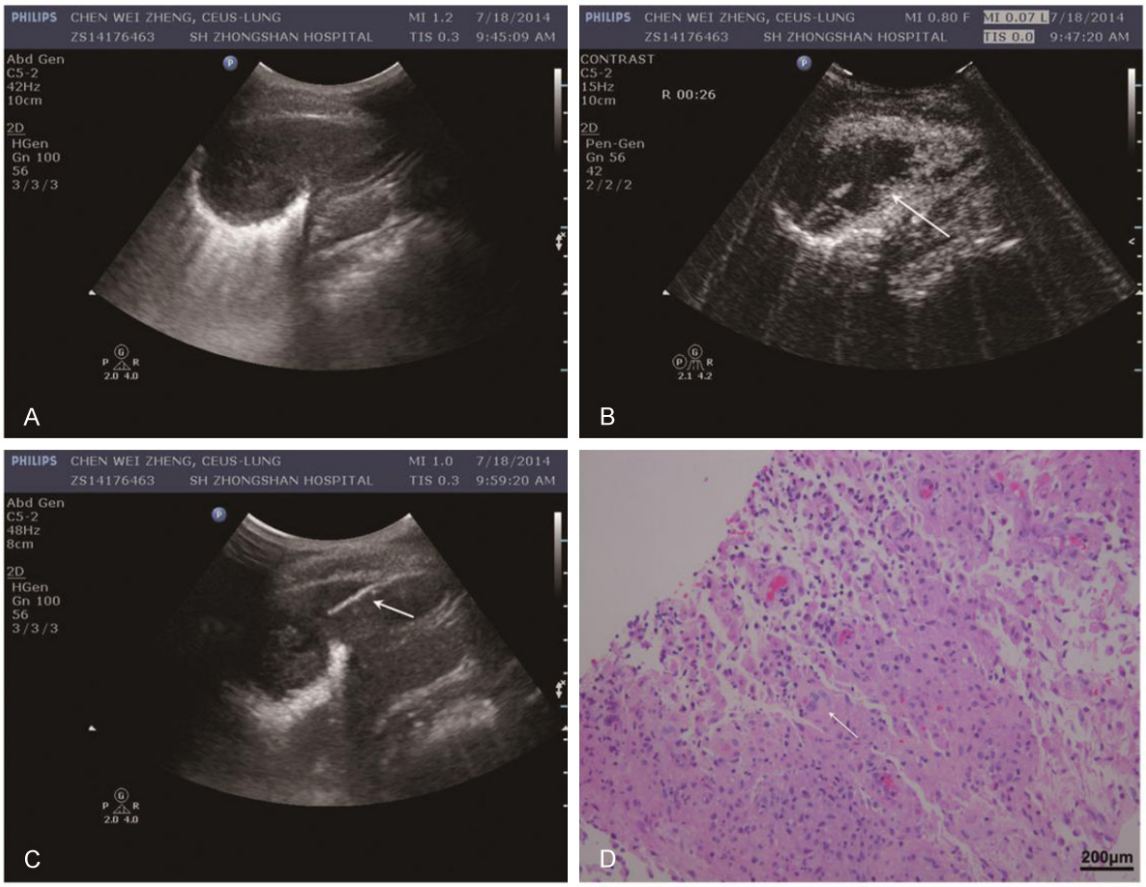


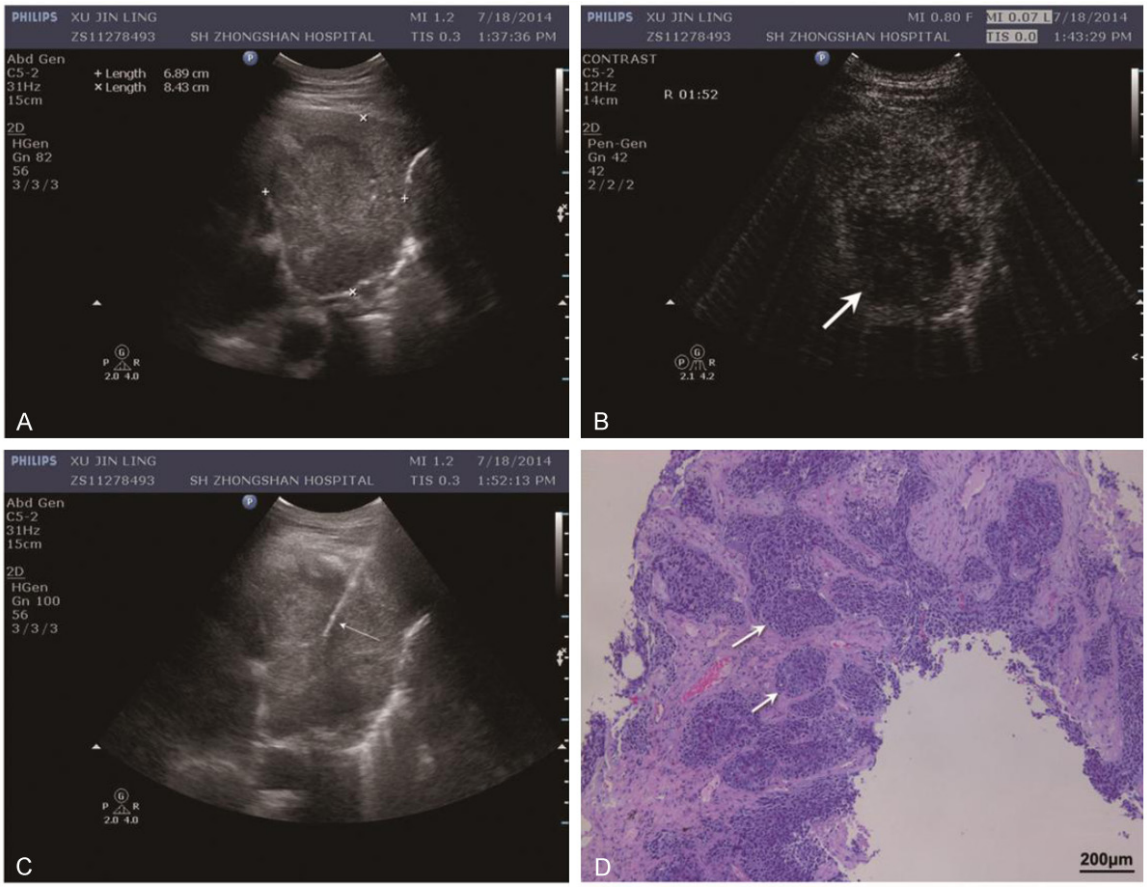

**Supplementary Figure 1.** Male, aged 74, with an irritating cough for one month. A. B-mode ultrasound showing a hypoechoiclump of 63×45 mm in the left lower lobe, with a regular shape and a little visible dark area inside (arrow). B. At 43 seconds after injection of the contrast agent, a large unenhanced area is revealed in the lump, which was significantly increased compared with before injection, and only a little active enhanced area (arrow) was visible in the upper left lobe. C. Under ultrasound guidance, biopsy was performed in the active area (arrow). D Pathological examination (×200) revealed a poorly differentiated lung adenocarcinoma (arrow).



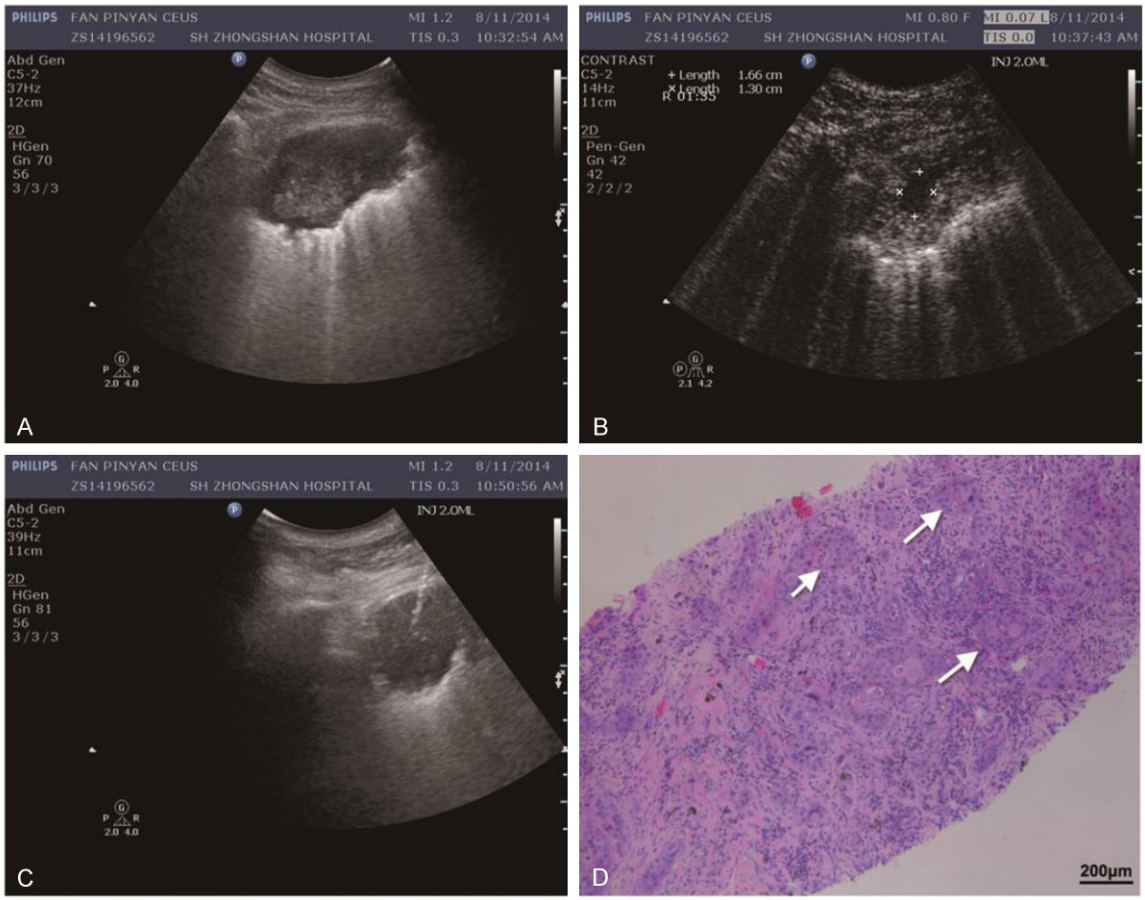

**Supplementary Figure 2.** Male, aged 64, with cough and expectoration for one month. A. B-mode ultrasound showing a hypoechoic lump in the left lower lobe, about 51×42 mm, with uniform echo and hyperechoic streaks (arrow). B. At 24 seconds after injection of the contrast agent, a uniform enhancement of the lump with visible enhancement of central pulmonary arteries and a streak-shape were visible (arrow). C. At 129 seconds after injection, no significant change in signal was visible, echo was still uniform, and central tubular hypoechoic bronchus was visible with a streak-shape (arrow). D. The biopsy confirmed pneumonia (arrow) (×200).




**Supplementary Figure 3.** Male, aged 25, with fever and cough for two weeks. A. B-mode ultrasound showed a hypoechoic lesion in the middle of the left lung, with an oval shape, uniform echo, and no obvious echo-free zone. B. At 26 seconds after injection of the contrast medium, an irregular unenhanced area (arrow) was revealed in the lump, and the tissues surrounding the lump were significantly enhanced. C. Under ultrasound guidance, a 16G biopsy gun was used to avoid lump necrosis, and biopsy was performed (arrow) in the active area around the lump. D. Pathology confirmed that the lesion was a lung granuloma and Langerhans cells could be seen (arrow) (×200).




**Supplementary Figure 4.** Male, aged 62, with anorexia and fatigue for 2 months. A. B-mode ultrasound showing a hypoechoiclump of 84×69 mm in the left upper lobe, with non-uniform echo and no obvious echo-free zone inside. B. At 112 seconds after injection of the contrast agent, a large unenhanced area was revealed below the lump. C. Under ultrasound guidance, a 16G biopsygun was used to puncture in the active area of the lump and harvest tissues (arrow). D. Pathological examination confirmed a small cell lung cancer (arrow) (×200).




**Supplementary Figure 5.** Male, aged 82, with bloody sputum. A. Ultrasound showed a hypoechoic oval and less homogeneous lump in the right lung, of about 60×40 mm. B. Ultrasound showed a small unenhanced area in the lump, of about 16×13 mm. C. CEUS-guided puncture was used to avoid the unenhanced area, and biopsy was performed in the active area. D. Pathology revealed a squamous cell lung carcinoma (×100), with typical squamous cell carcinoma nest (arrow).