Original Article

Enhanced ultrasound-guided versus non-enhanced ultrasound-guided percutaneous needle biopsy in tissue cellularity of lung malignancies: a propensity score matched study

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Background: Though ultrasound-guided percutaneous lung needle biopsy (US-PLNB) is a first-line small biopsy method for peripheral lung lesions, quality of cellularity in specimens obtained via US-PLNB is uncertain. This study investigated the accuracy, sensitivity, and cellularity of US-PLNB. It examined the ability of contrast-enhanced ultrasound (CEUS) to improve the effectiveness of US-PLNB.

Methods: We retrospectively analyzed all data of patients with subpleural lung lesions who underwent US-PLNB. The cellularity of US-PLNB from malignant lesions included the tumor cell number and proportion. The definition of high-quality cellularity (HQC) was concurrently achieving a tumor cell number ≥400 and a proportion ≥20%. The sensitivity, the actual numbers of tumor cell number/proportion, and the rate of HQC were calculated and compared between the CEUS and non-enhanced US groups after propensity score matching (PSM) with subgroup analyses by lesion size (small lesion ≤30 mm and large lesion >30 mm).

Results: A total of 345 patients undergoing 345 US-PLNBs were evaluated, with 3.7±1.1 of punctures on average. There were 201 malignant and 144 benign lesions with a mean size of 43.8±24.1 mm. Among the 201 malignant lesions, 124 cases underwent CEUS and 77 underwent non-enhanced US. The quantity of tumor cells, the proportion of tumor cells, and the rate of HQC in 201 cases of US-PLNB from malignant lesions were 2,862.1±2,288.0, 44.6%±24.5%, and 82.1% [95% confidence interval (CI): 76.6% to 87.1%], respectively. The quantity of tumor cells, the proportion of tumor cells, and rate of HQC were significantly higher in the CEUS group than that in the non-enhanced US group, both in the analysis of overall malignant lesions and in large malignant lesions (all $P<0.05$).

Conclusions: The US-PLNB has high sensitivity and thereby obtains HQC samples for subpleural lung malignant lesions. The CEUS helps improve the rate of HQC and tissue cellularity of lung malignancies.

Keywords: Contrast-enhanced ultrasound (CEUS); peripheral lung malignancy; needle biopsy; high-quality cellularity (HQC); propensity score matching (PSM)

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Introduction

Lung cancer is one of the most prevalent cancers, and its mortality ranks the first in all malignant tumors (1). Lung lesions are being increasingly detected because of the wide application of low-dose helical computed tomography (CT) (2-4). Histopathological examination is the gold standard for diagnosing lung lesions, and many tumor biomarker tests still require tissue samples, which can be taken from surgical specimens and small samples (5). A small sample biopsy in the diagnosis of lung malignancy, including ultrasound-guided percutaneous lung needle biopsy (US-PLNB), CT-guided PLNB, and bronchoscopic biopsy, is the main biopsy method for pathological diagnostics of lung malignancy for the reason that only minority of cases can be surgically removed (5,6). Additionally, advances in oncology and individualized treatment, such as gene-targeted therapy and immunotherapy, have brought significant benefits to the treatment of lung malignancy. Hence, small sample biopsy is playing an increasingly important role in the diagnosis and treatment of lung malignant lesions (7,8).

Due to the capability of US to clearly detect the subpleural lung lesions, US-PLNB is a first-line small biopsy method for peripheral lung lesions with high safety and diagnostic sensitivity for identifying malignant lung lesions (6,9-17). Therefore, with advances in oncology treatment, US-PLNB must satisfy auxiliary lung cancer detection requirements and not simply diagnose lesions pathologically. Consequently, obtaining samples with high-quality cellularity (HQC) is more important than ever due to the increasing requirements for US-PLNB. Goldhoff *et al.* reported that different biopsy needles significantly influenced the percentage of tumor cells in image-guided percutaneous liver lesions needle biopsy (18). Zhou *et al.* (19) also reported that cellularity of samples was significantly related to the diagnostic accuracy in US-guided mediastinal lesions needle biopsy. Nevertheless, few studies have reported on the quality of cellularity in specimens obtained via US-PLNB.

Further, it is necessary to examine whether US technology can be used to improve the quality of samples. Contrast-enhanced ultrasound (CEUS) adopts a blood pool contrast agent, which can be detected in microvessels with low flow, thus reducing Doppler artifacts to reflect the blood flow distribution more accurately (19,20). Like the liver, the lung also has a dual arterial blood supply, which may be helpful in the evaluation of peripheral pulmonary lesions (21). To date, CEUS has been widely used in the biopsy of various organs, including the lung, pancreas, and liver. However, it remains unclear whether CEUS can improve cellularity for US-PLNB.

This study investigated the accuracy, sensitivity, and cellularity of US-PLNB and analyzed the ability to use CEUS to improve the utility of US-PLNB. We present the following article in accordance with the Standards for Reporting Diagnostic accuracy studies (STARD) reporting checklist (available at https://qims.amegroups.com/article/view/10.21037/qims-22-119/rc).

Methods

The study was conducted in accordance with the Declaration of Helsinki, as revised in 2013. The study was approved by the Scientific Research Ethics Review Committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2018-14), and individual consent for this retrospective analysis was waived.

Study population

We retrospectively analyzed all data of patients treated at the First Affiliated Hospital of Guangzhou Medical University who underwent US-PLNB from January 2017 to July 2018. Participants were recruited consecutively. The inclusion criteria were as follows: age >16 years, the use of core needle cutting biopsy, and no antitumor treatment before US-PLNB. The exclusion criteria were as follows: missing data, extrapulmonary lesion, and repeated US-PLNB. Procedures that did not involve a puncture were also excluded.

Prebiopsy US evaluation and biopsy procedure

In the First Affiliated Hospital, Guangzhou Medical University, Guangzhou, all patients undergo a chest CT to evaluate lung lesions before a biopsy. Patients are preferentially considered for US-PLNB if the peripheral lesion was in contact with the pleural in the CT scan and could be detected under a B-mode ultrasound.

The US (MyLab 90; Esaote, Genoa, Italy) was performed with a low-frequency (2–5 MHz) convex transducer. Under non-enhanced US guidance, Doppler US was used to avoid thick blood vessels and suspected necrotic areas where the B-mode US showed an echoic area with a clear boundary and color doppler flow imaging (CDFI) displayed an absence of blood flow (19). However, when it was difficult to distinguish the necrotic area or atelectasis on the non-enhanced US and display the intratumoral blood flow on

Doppler US, and when the performance of non-enhanced US was obviously inconsistent with CT, CEUS then tended to be used according to the judgment of the operators. Under CEUS guidance, a 2.4-mL bolus of contrast agent (Sonovue®; Bracco, Milan, Italy) was injected. Using CEUS guidance, the non-enhanced area would be avoided. Further, among cases with different enhancement patterns in the lesion, the area showing relatively "slow-in and fast-out" enhancement for biopsy was selected (16,21,22). The CEUS was performed twice. The first CEUS was used to evaluate the overall situation of the lesion and determine the puncture path; the second CEUS was performed for real-time biopsy guidance with real-time gray-scale contrast tuned imaging in the planned path.

Two clinicians with at least 5 years of interventional operation experience cooperatively performed real-time US biopsies. Operator 1 was a sonographer who was responsible for US evaluation and guidance. Operator 2 used an 18 G core cutting needle with a specimen notch of 20 mm (MC1816, Bard Max Core; Bard Inc., Murray Hill, NJ, USA) to perform the biopsy under US guidance. Generally, 3–5 needle punctures were performed. However, if there were requirements for biopsy specimens to meet more auxiliary tests, 1–3 needle punctures were added if the patient was in a good condition.

Pathological diagnosis and cellularity evaluation

All specimens from US-PLNB were fixed in 10% formalin and sent for histopathological examination. All biopsy tissues underwent paraffin embedding and sectioning together. The samples were stained with hematoxylin and eosin (H&E). If the samples were suspected or diagnosed as malignant, further immunohistochemical tests were carried out to further classify the pathological type of the lesions.

The pathological diagnosis of all US-PLNBs was collected retrospectively. However, cellularity evaluation was performed later as part of the study. Pathologists were required to re-read all pathological sections with malignant pathological diagnoses to evaluate the cellularity. All pathological procedures were made through consultation between 2 pathologists with at least 5 years of experience blinded to the guidance method used.

An inadequate diagnosis of US-PLNB indicated that the specimens contained only necrotic tissue or skin and muscle tissue. A definite diagnosis was considered if the specimens from US-PLNB indicated malignancy. Benign diagnoses were confirmed by surgical pathology or a benign biopsy with at least 6 months of follow-up. If US-PLNB yielded an inadequate diagnosis or was inconsistent with the final diagnosis, it would be considered a failed diagnosis.

Cellularity evaluation of malignant US-PLNB specimens included calculation of the number and proportion of tumor cells. The number and proportion of tumor cells of failed US-PLNBs would be counted as 0. The proportion of tumor cells was calculated as the average ratio of the number of tumor cells to the number of nucleated cells counted in the specimen. The number of tumor cells was counted in 5 fields of 1 mm^2 each, and the means of these 5 values were calculated and then multiplied to obtain the total area of the specimen (23,24).

Variables and definitions

The satisfactory tumor cell number and proportion were defined as ≥400 and ≥20%, respectively (25,26). Additionally, the definition of HQC was concurrently achieving a tumor cell number ≥400 and proportion ≥20% (25,26). The lesion size was defined as the maximum length of a subpleural lesion perpendicular to the chest wall measured via axial CT of the mediastinal window. A lesion size less than 30 mm was defined as a small lesion, and greater than or equal to 30 mm was defined as a large lesion. Pleural contact length (PCL) was defined as the maximal contact length of the lesion that contacted the pleura in the largest area of the lesion measured via axial CT of the mediastinal window. The US-guided interventional procedures were performed by 3 operators with 5, 10, and 15 years of respective intervention experience.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). Chi-square or Fisher's exact tests were applied to compare the differences in categorical variables between groups. Differences in quantitative variables were determined using the independent sample *t*-test or the Mann-Whitney U test.

The overall diagnostic accuracy of US-PLNB and its sensitivity, actual number of tumor cell number/proportion, and the rate of HQC were calculated and compared between the CEUS and non-enhanced US groups. Subgroup analyses by tumor size were also conducted.

Propensity score matching (PSM) was conducted to decrease biases (27). The PSM was built upon a multivariate logistic regression analysis to predict the probability of each

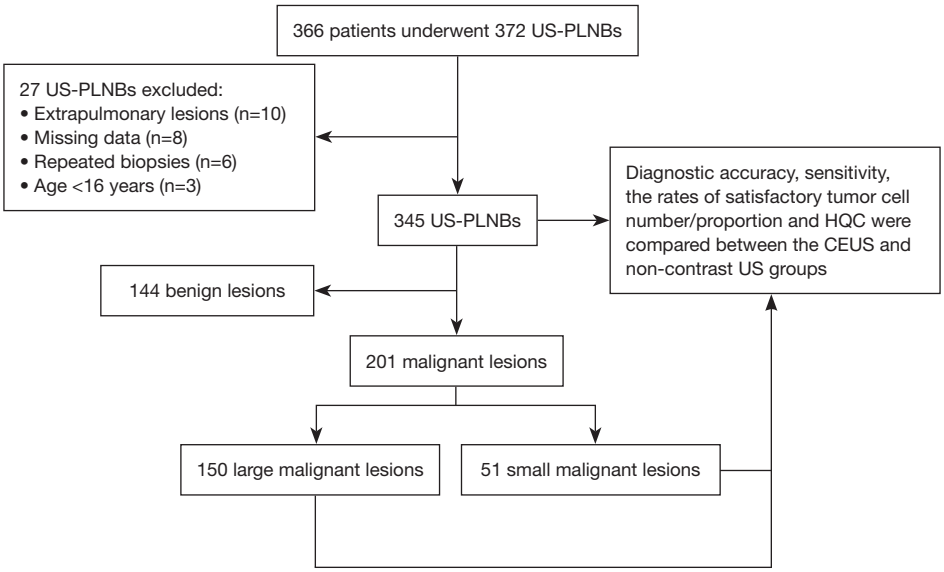

Figure 1 Flow diagram of the case selection and analysis process. US-PLNB, ultrasound-guided percutaneous lung needle biopsy; HQC, high-quality cellularity; US, ultrasound; CEUS, contrast-enhanced ultrasound.

individual patient being submitted to 1 of the 2 guiding methods based on covariates. The present study used PSM to adjust age, gender, location, size, PCL, number of biopsy punctures, and operator. We matched propensity scores 1:1 using the nearest neighbor method (without replacement) using a caliper size of 0.05 standard deviation (SD). In all analyses, P<0.05 indicated statistical significance.

Results

A total of 366 patients underwent 372 US-PLNBs during the study period, of which 27 were excluded (*Figure 1*). Ultimately, 345 patients with 345 US-PLNBs were evaluated, including 190 cases with CEUS guidance and 155 cases with non-enhanced US guidance (*Table 1*). The average number of punctures in 345 US-PLNBs was 3.7±1.1 (range, 1 to 8). The patients consisted of 252 males and 93 females, with a mean age of 58.1±14.6 years (range, 17 to 92 years). A total of 156 and 189 lesions were located in the left and right thorax, respectively, with the mean lesion size of 43.8±24.1 mm (range, 7.4 to 124.2 mm). The final diagnostic results of 345 cases were 201 malignant lesions and 144 benign lesions (*Table 2*).

Accuracy and complications of overall US-PLNB

The use of US-PLNB resulted in 315 cases of correct diagnosis and 30 cases of failed diagnosis. The 30 cases of failed diagnosis included 11 cases of inadequate diagnosis and 19 cases of false negative biopsy. There were 50 complications, including 26 cases of hemoptysis, 12 cases of pleural reaction, 8 cases of pneumothorax, and 4 cases of intralesional hemorrhage or hemothorax. The overall accuracy and complication rates were 91.3% [95% confidence interval (CI): 88.1% to 93.9%] and 14.5%, respectively.

Before PSM, the lesion size, PCL, and the number of punctures were significantly greater in the CEUS group than in the non-enhanced US group (all P<0.05). However, the 2 groups had no significant differences in age, gender, lesion location, or operator. In addition, although the diagnostic accuracy was higher and the complication rate was lower in the CEUS group (92.6% *vs.* 89.7% and 14.2% *vs.* 14.8%), the differences were not statistically significant.

After PSM, there were no significant differences in lesion size, PCL, the number of punctures, age, gender, location, or operator between the 2 groups. There were also no significant differences in accuracy or complication rate.

Analysis of sensitivity and cellularity of lesions

Among the 201 malignant lesions, there were 19 cases of failed diagnosis and 182 cases of correct diagnosis. The sensitivity of US-PLNB in terms of malignant lesions was 90.5% (95% CI: 86.1% to 94.5%). The quantity of tumor

Table 1 Patient demographics

Demographics	CEUS (n=190)	Non-enhanced US (n=155)	P value
Age (years)*	59.3±12.9	56.7±16.3	0.277
Gender			0.465
Male	142	110	
Female	48	45	
Location			1.000
Left thorax	86	70	
Right thorax	104	85	
Lesion size (mm)*	50.0±25.3	36.2±20.2	<0.001†
PCL (mm)*	57.6±26.6	43.1±27.6	<0.001†
Pathology			0.004†
Benign	66	78	
Malignant	124	77	
Operators			0.056
1	62	66	
2	20	21	
3	108	68	
Number of punctures*	3.8±1.1	3.5±1.0	0.003†
Accuracy			0.344
No	14	16	
Yes	176	139	
Complication			0.879
No	163	132	
Yes	27	23	

*, data are presented as means ± standard deviations; †, statistically significant (P<0.05). US, ultrasound; CEUS, contrast-enhanced ultrasound; PSM, propensity score matching; PCL, pleural contact length.

cells and proportion of tumor cells in 201 US-PLNBs were 2,862.1±2,288.0 and 44.6%±24.5%, respectively. A satisfactory tumor cell number was obtained in 173 cases, a satisfactory tumor cell proportion was achieved in 169 cases, and 165 samples of US-PLNB had HQC. The overall US-PLNBs had 86.1% (95% CI: 81.1% to 90.5%), 84.1% (95% CI: 78.6% to 89.1%), and 82.1% (95% CI: 76.6% to 87.1%) in rates of the satisfactory tumor cell number, the satisfactory tumor cell proportion, and HQC, respectively.

Among the 201 cases of malignancy, 124 cases underwent CEUS and 77 underwent the non-enhanced US. Before PSM, the lesion size, PCL, and the number of punctures were significantly greater in the CEUS group (all P<0.05). There were no significant differences in other covariates between 2 groups. The quantity of tumor cells in the CEUS group was significantly higher than that in the non-enhanced US group (3,202.0±2,227.8 *vs.* 2,314.7±2,291.7; P=0.001). However, although the rate of satisfactory tumor cells in the CEUS group was slightly higher than that in the non-enhanced US group, there was no significant difference between the 2 groups (89.5% *vs.* 80.5%; P=0.073). Further, the proportion of tumor cells in the CEUS group was also significantly higher than that in the non-enhanced US group (47.2%±24.6% *vs.* 40.4%±23.9%; P=0.038), while there was no significant difference between the two groups on the rate of satisfactory tumor cell proportion (86.3%

Table 2 The final diagnosis between the CEUS and non-enhanced US group

Diagnosis	CEUS (n=190)	Non-enhanced US (n=155)	Total (n=345)
Malignant diagnosis	124	77	201
Lung adenocarcinoma	58	36	94
Lung squamous cell carcinoma	28	15	43
Non-small lung cell carcinoma (not otherwise specified)	6	4	10
Small cell lung carcinoma	12	7	19
Other carcinoma	20	15	35
Benign diagnosis	66	78	144
Nonspecific inflammation	44	44	88
Cryptococcus	6	8	14
Tuberculosis	6	13	19
Other specific benign	10	13	23

US, ultrasound; CEUS, contrast-enhanced ultrasound.

vs. 80.5%; P=0.277). Nevertheless, the rate of HQC in the CEUS group was significantly higher than that in the non-enhanced US group (86.3% *vs.* 75.3%; P=0.049). The details are shown in *Table 3*.

After PSM, there were no significant differences in any covariates between the 2 groups. However, the quantity of tumor cells, the proportion of tumor cells, the rate of HQC, and the rate of satisfactory tumor cell number were significantly higher in the CEUS group (all P<0.05). Nevertheless, there were still no significant differences in sensitivity or rates of satisfactory tumor cell proportion. The details are shown in *Table 3*.

Subgroup analysis according to the size of malignant lesions

In subgroup analyses of 150 large malignant lesions, before PSM, the lesion size, PCL, and the number of punctures were also significantly greater in the CEUS group (all P<0.05). The quantity of tumor cells in the CEUS group was significantly higher than that in the non-enhanced US group (3,131.8±2,154.2 *vs.* 2,253.6±2,347.5; P=0.004), and the rate of satisfactory tumor cell number was also significantly higher in the CEUS group than that in the non-enhanced US group (88.9% *vs.* 76.5%; P=0.046). The proportion of tumor cells in the CEUS group was also significantly higher than that in the non-enhanced US group (46.5%±24.0% *vs.* 37.7%±23.5%; P=0.023), but there was no significant difference in the rate of tumor cell proportion between the 2 groups (85.9% *vs.* 78.4%; P=0.248). Nevertheless, the rate of HQC in the CEUS group was significantly higher than that in the non-enhanced US group (85.9% *vs.* 72.5%, respectively; P=0.048). After PSM, there were no statistically significant differences in any covariates between the 2 groups. The quantity of tumor cells, the proportion of tumor cells, and the rate of HQC were still significantly higher in the CEUS group (all P<0.05). Nevertheless, there were still no significant differences in the sensitivity, the rates of satisfactory tumor cell proportion, and the rates of satisfactory tumor cell number. The details are shown in *Table 4*.

In subgroup analyses of 51 small malignant lesions, there were no significant differences in covariates between the 2 groups, except in the number of punctures, which was significantly higher in the CEUS group (all P<0.05). After PSM, there were no significant differences in any covariates between the 2 groups. The sensitivity, the quantity of tumor cells, the proportion of tumor cells, the rate of satisfactory tumor cell number, the rate of satisfactory tumor cell proportion, and the rate of HQC were not significantly different between the 2 groups before or after PSM.

Discussion

Currently, US-PLNB is widely used and is recommended as the first-line method for small sample biopsies of peripheral subpleural lung lesions (5,10). The tissue quality

Table 3 The difference in overall malignant lesion characteristics, diagnostic sensitivity, and cellularity between non-enhanced US and CEUS before and after PSM

Variables	Before PSM			After PSM		
	CEUS (n=124)	Non-enhanced US (n=77)	P value	CEUS (n=69)	Non-enhanced US (n=69)	P value
Age (years)*	62.8±10.9	64.1±12.3	0.372	63.3±11.4	64.1±12.5	0.617
Gender			0.449			0.848
Male	93	54		51	50	
Female	31	23		18	19	
Location			0.617			0.863
Left thorax	56	32		29	30	
Right thorax	68	45		40	39	
Lesion size (mm)*	53.3±24.7	40.3±23.2	<0.001†	46.7±24.4	43.4±22.5	0.481
PCL (mm)*	60.2±25.5	47.5±29.5	<0.001†	52.3±23.5	51.0±29.1	0.470
Operators			0.148			0.284
1	41	26		16	23	
2	14	16		12	14	
3	69	35		41	32	
Number of punctures*	3.9±1.0	3.5±1.0	0.018†	3.5±0.8	3.6±0.9	0.422
Diagnostic sensitivity			0.721			0.366
No	11	8		4	8	
Yes	113	69		65	61	
Tumor cells number	3,202.0±2,227.8	2,314.7±2,291.7	0.001†	3,088.4±2,328.6	2,338.2±2,280.9	0.028†
Tumor cells proportion	47.2±24.6	40.4±23.9	0.038†	52.1±25.3	39.1±24.2	0.002†
Satisfaction of tumor cells number			0.073			0.026†
No	13	15		5	14	
Yes	111	62		64	55	
Satisfaction of tumor cells proportion			0.277			0.110
No	17	15		8	15	
Yes	107	62		61	54	
HQC			0.049†			0.029†
No	17	19		8	18	
Yes	107	58		61	51	

*, data are presented as means ± standard deviations; †, statistically significant (P<0.05). US, ultrasound; CEUS, contrast-enhanced ultrasound; PSM, propensity score matching; PCL, pleural contact length; HQC, high-quality cellularity.

and utility of US-PLNB should be further investigated with the development of precision therapy. Therefore, we investigated the tissue cellularity of US-PLNB in lung malignancies and explored the value of CEUS. Currently, the HQC of US-PLNB should be required for most clinical auxiliary methods (8,25,26,28,29). However, different auxiliary tests and test methods have different requirements on the cellularity of samples. Nevertheless, the consensus

Table 4 The difference in large malignant lesion characteristics, diagnostic sensitivity, and cellularity between non-enhanced US and CEUS before and after PSM

Variables	Before PSM			After PSM		
	CEUS (n=99)	Non-enhanced US (n=51)	P value	CEUS (n=48)	Non-enhanced US (n=48)	P value
Age (years)*	62.9±10.9	65.5±11.6	0.176	63.9±12.6	64.8±11.6	0.980
Gender			0.654			0.473
Male	77	38		38	35	
Female	22	13		10	13	
Location			0.380			1.000
Left thorax	43	26		24	23	
Right thorax	56	25		24	25	
Lesion size (mm)*	61.1±20.1	52.3±19.1	0.010†	56.7±20.4	53.4±19.1	0.442
PCL (mm)*	68.0±21.5	59.0±28.1	0.005†	60.5±17.8	59.3±28.7	0.393
Operators			0.115			0.334
1	34	15		9	15	
2	12	13		10	10	
3	53	23		29	23	
Number of punctures*	3.9±1.1	3.8±0.8	0.510	3.7±1.2	3.8±0.8	0.359
Diagnostic sensitivity			0.605			0.486
No	9	6		3	6	
Yes	90	45		45	42	
Tumor cells number	3,131.8±2,154.2	2,253.6±2,347.5	0.004†	2,916.7±2,232.8	2,207.0±2,406.8	0.046†
Tumor cells proportion	46.5±24.0	37.7±23.5	0.023†	52.2±24.7	36.7±23.7	0.002†
Satisfaction of tumor cells number			0.046†			0.053
No	11	12		4	12	
Yes	88	39		44	36	
Satisfaction of tumor cells proportion			0.248			0.181
No	14	11		6	11	
Yes	85	40		43	37	
HQC			0.048†			0.044†
No	14	14		6	14	
Yes	85	37		42	34	

*, data are presented as means ± standard deviations; †, statistically significant (P<0.05). US, ultrasound; CEUS, contrast-enhanced ultrasound; PSM, propensity score matching; PCL, pleural contact length; HQC, high-quality cellularity.

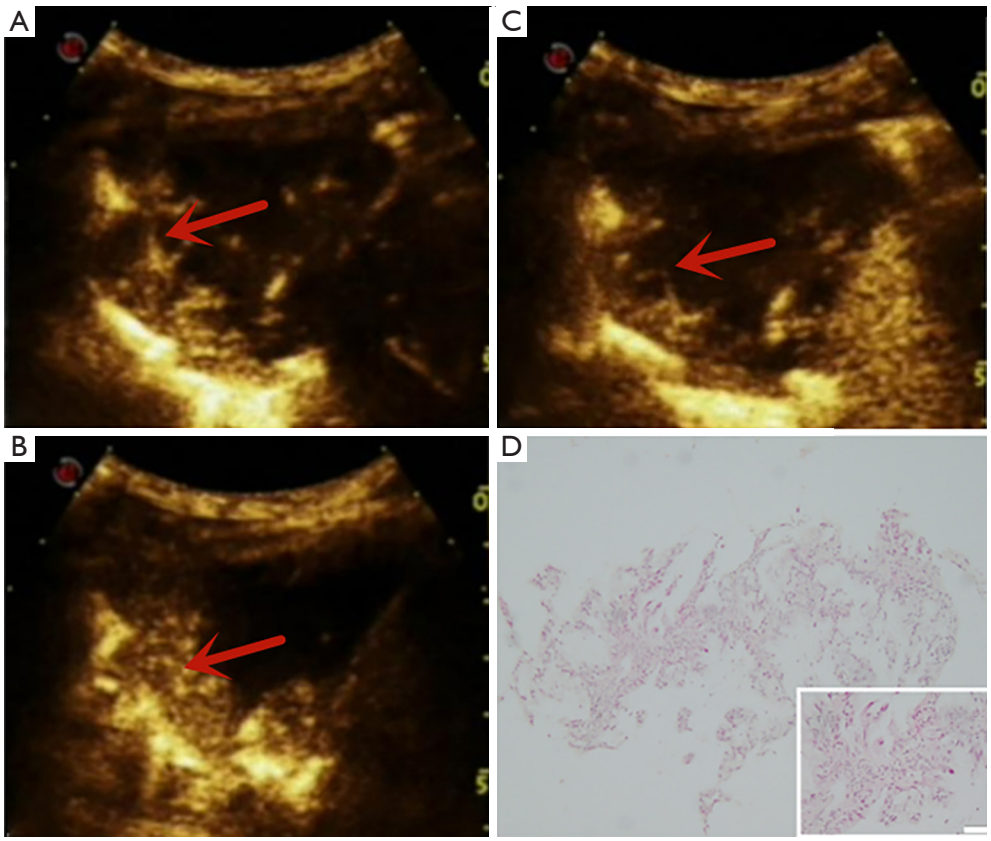

Figure 2 A 54-year-old male patient with a lesion in the right lung. (A-C) CEUS showed no enhancement and "slow-in and fast-out" enhancement (red arrow) in the lesion. CEUS was used to locate the "slow-in and fast-out" enhancement area (red arrow) in real-time for biopsy. (D) The pathological diagnosis was lung adenocarcinoma. The number of tumor cells was >400 and the proportion of tumor cells was >20% in the specimen (H&E; original magnification 100×; inset magnification 400×). CEUS, contrast-enhanced ultrasound; H&E, hematoxylin and eosin.

and guidelines for gene mutation testing in non-small cell lung cancer recommend at least 200–400 tumor cells, and a tumor percentage as low as 20% (25,26), which can meet the common auxiliary detection requirements in the clinic. Therefore, in the present study, we defined a satisfactory tumor cell number as ≥400 and a satisfactory tumor cell proportion as ≥20%; the HQC of US-PLNB had to concurrently meet both of those requirements. We found that the quantity of tumor cells and proportion of tumor cells in 345 US-PLNBs were 2,862.1±2,288.0 and 44.6%±24.5%, respectively. The overall US-PLNBs had 86.1%, 84.1%, and 82.1% in rates of satisfactory tumor cell number, satisfactory tumor cell proportion, and HQC, respectively. Further, the rate of HQC was 86.3% in the CEUS group, and 75.3% in the non-enhanced US group, respectively. Thus, the US-PLNB had a high rate of HQC which can fulfill most of the requirements for auxiliary testing of malignant lung lesions in clinical work. to the best of our knowledge, this was the first study to investigate the quality of cellularity in specimens obtained by US-PLNB.

In the present study, like a previous report (12), although the diagnostic accuracy was slightly higher in the CEUS group than in the non-enhanced US group, the difference was not significant. However, we found the quantity of tumor cells, the proportion of tumor cells, and the rate of HQC was significantly higher in the CEUS group (*Figure 2*). A prospective molecular triage study that included 77 lung cases showed that cellularity was significantly higher with CEUS-guided biopsy than with non-enhanced images (30). In addition, Zhou *et al.* (19) reported that CEUS improved the yield of conclusive histological diagnoses of mediastinal masses compared to non-enhanced US because of the increased cellularity of samples. Although the conclusions of these previous studies are similar to some of our

viewpoints, their analysis of cellularity focused mainly on the proportion of tumor cells, and the number of lung samples was small. In our study, we investigated both the number and proportion of tumor cells concurrently.

In subgroup analyses of large malignant lesions, CEUS also significantly improved the quantity of tumor cells, the proportion of tumor cells, and the rate of HQC; however, in small malignant lesions, it did not. Wang *et al.* (13) reported that CEUS was helpful for biopsy of large lesions (≥3 cm), with advantages in identifying necrosis and atelectasis, but it was not helpful for small lesions (<3 cm). This is similar to our findings. There are several possible reasons for this. First, the extent and proportion of necrosis may increase with the size of the lung lesion. Guo *et al.* (11) reported that the proportion of intratumoral necrosis increased with the increase of lesion size. Therefore, although CEUS can be sensitive for identifying necrotic areas showing no enhancement, its advantages for identifying necrosis would not be reflected if the extent of necrosis was not obvious inside a small lesion. Second, atelectasis may be more likely to occur in large lesions (16). Therefore, the use of CEUS significantly affects the results of large lesions samples.

It is worth noting that the present study was a non-randomized retrospective study, but we used PSM to eliminate confounding factors between the 2 groups. Before matching, although the quantity of tumor cells, the proportion of tumor cells, and the rate of HQC were significantly higher in the CEUS group, there were also significant differences between the 2 groups in lesion size, PCL, and the number of punctures. The size and PCL had previously been reported to influence the accuracy and sensitivity of US-PLNB (11,17). Tumor cell number may also increase with the number of effective punctures. These factors may interfere with the evaluation of contrast efficiency. However, after PSM, all covariates reached a relative balance with no significant differences between the 2 groups. In addition, the quantity of tumor cells, the proportion of tumor cells, and the rate of HQC still significantly differed between the 2 groups after matching. to the best of our knowledge, this was also the first study to apply PSM to comparative analyses of CEUS-PLNB and non-enhanced US-PLNB.

This study also had some limitations. We only defined a minimum cellularity index that could meet most clinical requirements. Some of the lesions in this study were not evaluated by enhanced imaging, and non-enhanced imaging was not considered to evaluate necrosis sufficiently, so we did not analyze the extent and proportion of necrosis inside lesions. This was a single-center study, and the operators who performed puncture and guidance all had extensive experience. This situation may not be applicable to some other centers.

In conclusion, US-PLNB has high diagnostic accuracy and sensitivity for lung cancer and obtained HQC samples from lung cancer lesions. Further, CEUS is also beneficial for improving the quantity of tumor cells, the proportion of tumor cells, and the rate of HQC samples overall, particularly for large malignant lesions.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://qims.amegroups.com/article/view/10.21037/qims-22-119/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://qims.amegroups.com/article/view/10.21037/qims-22-119/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Scientific Research Ethics Review Committee of the First Affiliated Hospital of Guangzhou Medical University (No. 2018-14), and individual consent for this retrospective analysis was waived.

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