Improved diagnostic yield of transbronchial lung biopsy in peripheral pulmonary lesions using a combination of endobronchial ultrasound and rapid on-site evaluation

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Abstract

Objectives: To evaluate the value of rapid on-site evaluation (ROSE) during radial probe endobronchial ultrasound transbronchial lung biopsy (rpEBUS-TBLB) for peripheral pulmonary lesions (PPLs).

Methods: One hundred and six patients with PPLs who received rpEBUS-TBLB were enrolled in this study. One specimen was immediately examined by ROSE and the other was sent to the central laboratory for cytologic diagnosis. The results of ROSE were compared with those of pathological diagnosis.

Results: The diagnostic accuracy, sensitivity, and specificity of ROSE during rpEBUS-TBLB for PPLs were 82.1%, 89.6%, and 77.1%, respectively. The procedure times and number of biopsies were less for procedures when ROSE was positive compared with those when ROSE was negative (procedure time: 20.5 ± 7.9 vs. 28.3 ± 7.6 minutes; number of biopsies: 1.6 ± 0.9 vs. 2.8 ± 0.6 times). No serious procedural complications were observed.

Conclusions: ROSE has value for diagnosing PPLs during rpEBUS. It can reduce procedure time, number of biopsies, and complications. ROSE combined with rpEBUS is an effective and safe method for the diagnosis of PPLs.

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Introduction

With the development of imaging technology, the multi-slice spiral computed tomography (CT) examination of the chest has become a common diagnostic test, and the detection rate of peripheral pulmonary lesions (PPLs) has gradually increased. However, because of the complex causes of the lesions, they often lack specific imaging features and it is not easy to distinguish benign from malignant lesions in the clinic. In recent years, auxiliary techniques have been used, including virtual bronchoscopy, magnetic navigation, radial ultrasound, and ultra-fine bronchoscopy, among others.[1] Endobronchial ultrasonography (EBUS) has become an important examination method for peripheral lung diseases because of its distinct focus, high safety, and low cost; however, it is still difficult to determine whether a satisfactory specimen has been obtained. Being able to judge when the target specimen has been obtained and whether the specimen is sufficient and appropriate is also important for the diagnosis.

Rapid on-site evaluation (ROSE) is a real-time, rapid cytological interpretation technique that can quickly indicate sample characteristics and evaluate their quality.[2] In recent years, many reports have indicated that ROSE has improved the diagnostic rate and reduced complications associated with transbronchial needle aspiration, which are valuable in clinical applications. However, there are few reports on its application for PPLs.[3,4] The purpose of this study was to explore the value of ROSE during radial probe EBUS transbronchial lung biopsy (rpEBUS-TBLB) in improving the diagnostic yield of PPLs.

Materials and methods

This study was approved by the Ethics Committee of Nanjing Chest Hospital and was carried out in accordance with national law and the current revised Declaration of Helsinki. Written informed consent was obtained from all participants in the study.

Patients

This was a retrospective study. From February 2017 to September 2019, 106 patients with PPLs were treated in the Nanjing Chest Hospital. All patients underwent rpEBUS-TBLB with guide sheath (rpEBUS-GS-TBLB). Inclusion criteria were as follows: patients with undiagnosed peripheral lung lesions found by chest CT; patients with undiagnosed lesions not found by electronic bronchoscopy but found by small ultrasound probe; patients with normal routine blood work, electrocardiogram, clotting time, and liver and kidney function tests; and no contraindication to general anesthesia and bronchoscopy. The patients agreed to EBUS-TBLB and met medical ethics requirements. Exclusion criteria were as follows: patients with bleeding tendency, severe cardiorespiratory insufficiency, intolerance to or noncompliance with bronchoscopy; or patients without lesions detected by rpEBUS.

Performance of rpEBUS-TBLB

Preoperative chest CT was performed to assess the size and location of lesions and to identify the bronchi (Figure 1A). All procedures used a BF-P260F bronchoscope (Olympus, Tokyo, Japan) with 2.8-mm working channel. The radial EBUS probe had a diameter of 2.0 mm (UM-S20-17S; Olympus) and the guide sheath measured 2.2 mm in diameter (K201 kit; Olympus). All patients had fasted for 6 hours and abstained from liquids for 8 hours before the operation. With the patient under conscious sedation, 2% lidocaine was nebulized for topical anesthesia. Heart rate, peripheral oxygenation, and blood pressure were monitored during the procedure. After the bronchoscope was advanced through the nose and reached the location of the lesion identified prior to the procedure, the sheathed EBUS probe was guided through the bronchoscope channel in a gradual approach to the lesion to obtain the EBUS image (Figure 1B). The probe and guide sheath were adjusted according to the EBUS image during the procedure, and the position of the lesion around the probe was selected until the characteristic ultrasound signal indicating the presence of solid lesions was located. Once the appropriate EBUS image was found, an assistant secured the bronchoscope to the patient's nasal cavity, fixed the guide sheath externally to the entrance of the bronchoscope biopsy channel, and then removed the probe from the guide sheath, leaving the guide sheath in place. A bronchial brush (JHK-BC-18, Jiuhong Medical Instrument Co. Ltd., Jiangsu, China) was used to guide the sheath to obtain the pathological specimen. The specimen was evenly and rapidly smeared on three slides. One specimen was immediately examined by ROSE and the other was sent to the central laboratory for cytologic diagnosis. When ROSE was positive (Figure 1C and 1D), bronchial brushing was completed. If the sample was of no diagnostic value, the bronchial brushing was performed again at the original site or at another site. TBLB was performed at each site after bronchial brushing without fluoroscopy.

Performance of ROSE and sample processing

The brushing specimens smeared on glass slide were used for on-site evaluation with Diff-Quick staining. The specimen was fixed and stained in Diff-Quick A solution for 20 s, slowly soaked in phosphate to rinse off the Diff-Quick A solution, gently dried, and then stained in Diff-Quick B solution for 10 s. Then, the cells were washed, dried, and examined under the microscope by a certified cytology expert to make a preliminary cytological diagnosis. All slides and tissues were sent to the pathology department for destaining followed by hematoxylin and eosin staining and immunohistochemistry. Two senior pathologists reviewed the slides and confirmed the final diagnosis. If the results were inconsistent, a third pathologist discussed and decided the final pathological results as the "gold standard."

Outcome measures

EBUS-TBLB does not result in a definitive diagnosis and results must be confirmed by other methods. The non-malignant pathological results were confirmed as follows: CT-guided percutaneous needle biopsy, video-assisted thoracoscopic surgery, or other surgical biopsy. If patients without a definitive diagnosis did not consent to further examination, they were followed for 6 months.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Using the method of sample size estimation, the required sample size was calculated to be 106 cases. Measurement data
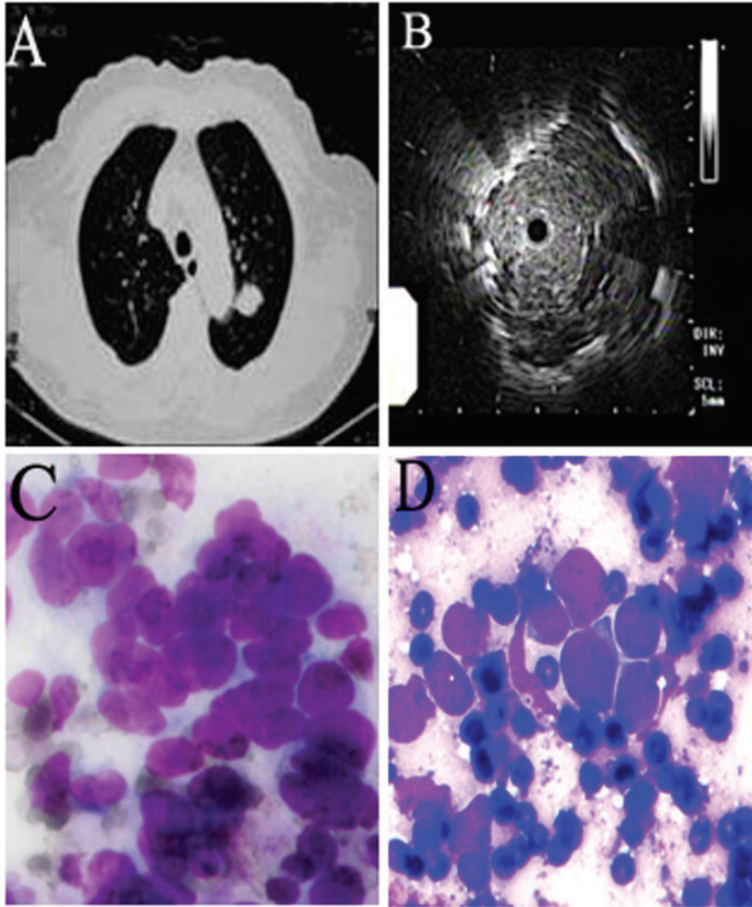
Figure 1. (a) Chest computed tomography scan showing a lesion involving the right lower lobe; (b) typical endobronchial ultrasound images of lesions; (c) adenocarcinoma diagnosed by rapid on-site evaluation (ROSE) using Diff-Quick staining; (d) small-cell lung cancer diagnosed by ROSE using Diff-Quick staining.

are expressed as means ± standard deviations, and count data are expressed as percentages. The unpaired t-test was used to analyze continuous variables, and the χ^2 test was applied to compare percentages. $P < 0.05$ was considered significant.

Results

Clinical characteristics

The study patients had an average age of 60.7 ± 10.8 years (range: 32–76 years).

The median lesion size was 20.2 ± 9.8 mm (8–30 mm). Seventeen cases were located in the right upper lobe, 35 cases in the right middle lobe, 28 cases in the right lower lobe, 12 cases in the left upper lobe, and 14 cases in the left lower lobe. Of the EBUS images, 66 (62.3%) were located in the center of the lesions and 34 (32.1%) were adjacent to the lesions. No ultrasound signal was found in 6 lesions (5.7%). Seventy-one cases were diagnosed as malignant and 35 cases as benign (Table 1).

Table 1. Clinical characteristics of patients undergoing rpEBUS-GS-TBLB with ROSE.

Characteristic	Value
Patients (number)	106
Sex	
Male	64 (60.4%)
Female	42 (39.6%)
Age (years)	60.7 ± 10.8 (32–76)
Lesion size (mm)	20.2 ± 9.8 (8–30)
Location of lesions on coronal CT	
One-third of central	50 (47.2%)
One-third of middle	30 (28.3%)
One-third of peripheral	26 (24.5%)
Lesion location	
Right upper lobe	17 (16.0%)
Right middle lobe	35 (33.0%)
Right lower lobe	28 (26.4%)
Left upper lobe	12 (11.3%)
Left lower lobe	14 (13.2%)
EBUS image	
Within	66 (62.3%)
Adjacent to	34 (32.1%)
Invisible	6 (5.7%)
Final diagnosis	
Malignant disease	
Adenocarcinoma	54 (50.9%)
Squamous cell carcinoma	10 (9.4%)
Small cell lung cancer	4 (3.8%)
Metastatic tumor	3 (2.8%)
Benign disease	
Tuberculosis	12 (11.3%)
Pneumonia	10 (9.4%)
Pulmonary aspergillosis	4 (3.8%)
Pulmonary hamartoma	6 (5.7%)
Organizing pneumonia	3 (2.8%)

rpEBUS-GS-TBLB, radial probe endobronchial ultrasound transbronchial lung biopsy with guide sheath; CT, computed tomography; ROSE, rapid on-site evaluation.

Association of ROSE with pathologic results of rpEBUS-TBLB

The ROSE results and final pathologic results of rpEBUS-TBLB differed in 19 cases. Sixty-seven cases were positive by rpEBUS-TBLB but 7 of those were negative by ROSE. Similarly, 39 cases were negative by rpEBUS-TBLB but 12 of those were positive by ROSE. In the diagnosis of PPLs by ROSE combined with rpEBUS-TBLB, the diagnostic accuracy, sensitivity,

Table 2. Comparison of ROSE results with rpEBUS-GS-TBLB in the pathologic diagnosis of peripheral pulmonary lesions.

ROSE	EBUS-GS-TBLB results		
	Positive	Negative	Total
Positive	60	12	72
Negative	7	27	34
Total	67	39	106

Accuracy = 82.1%, sensitivity = 89.6%, specificity = 77.1%, positive predictive value = 83.3%, negative predictive value = 79.4%.
ROSE, rapid on-site evaluation; rpEBUS-GS-TBLB, radial probe endobronchial ultrasound transbronchial lung biopsy with guide sheath.

specificity, positive predictive value, and negative predictive value were 82.1%, 89.6%, 77.1%, 83.3%, and 79.4%, respectively (Table 2).

Association of ROSE with final pathological diagnosis

In total, 106 lesions examined by rpEBUS were followed up to obtain the final pathological diagnosis. Of these, 71 were malignant and 35 were benign. The diagnostic accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of ROSE in the diagnosis of malignant lesions was 91.5%, 88.7%, 97.1%, 98.4%, and 81.0%, respectively (Table 3).

Effect of rpEBUS-TBLB on diagnostic yield

In 106 cases of PPLs, 100 cases were detected and located by rpEBUS, and 6 cases were not detected by rpEBUS. The total diagnostic yield of rpEBUS-TBLB was 73.6%; the diagnostic yield of patients with the ultrasound probe within the lesion was significantly higher than that of patients with the ultrasound probe adjacent to the lesion (87.9% vs. 58.8%; $P < 0.01$). The diameter of the PPL also affected diagnostic yield. Of 46 cases with lesions 8 to 20

Table 3. Comparison of ROSE results with the final pathologic diagnosis of peripheral pulmonary lesions.

ROSE	Final diagnosis		
	Malignant	Benign	Total
Malignant	63	1	64
Benign	8	34	42
Total	71	35	106

Accuracy = 91.5%, sensitivity = 88.7%, specificity = 97.1%, positive predictive value = 98.4%, negative predictive value = 81.0%.
ROSE, rapid on-site evaluation.

mm in diameter, 28 cases (60.9%) were diagnosed. The diagnostic yield was 83.3% with lesions of 20 to 30 mm in diameter ($P < 0.05$). Diagnostic yield varied with the location of the lesion. Only 16 of 29 cases with bilateral upper lobe lesions were diagnosed (diagnostic yield of 55.2%), which was lower than for lesions in the right middle lobe and bilateral lower lobe ($P < 0.05$). The feature of the nodules also affected the diagnostic yield: that of solid nodules was significantly higher than that of ground-glass nodules ($P < 0.05$) (Table 4).

Comparison of number of biopsies and procedure times

When ROSE was positive, the number of biopsies was 1.6 ± 0.9, and procedure times averaged 20.5 ± 7.9 minutes; when ROSE was negative, the number of biopsies was 2.8 ± 0.6, and procedure times averaged 28.3 ± 7.6 minutes (both $P < 0.05$) (Table 5).

Complications

The main complications were hemoptysis and chest pain. No severe complications such as pneumothorax, severe hypoxemia, or arrhythmia occurred. Hemoptysis occurred in 23 cases (21.7%), but the amount of hemoptysis was small (<20 mL). During the procedure, bleeding stopped after a topical spray of 1:1000 iced saline, epinephrine, or thrombin through the bronchoscope biopsy channel. Twelve patients (11.3%) had chest pain, but the pain was mild and tolerable, and the symptoms gradually resolved without treatment.

Discussion

The 5-year survival rate for early detection of malignant pulmonary nodules after surgical resection is greater than 80%, whereas that for advanced lung cancer is less than 18%.[5] Therefore, when suspicious malignant nodules are found on CT, it is very important to use effective diagnostic methods for early diagnosis.

EBUS can find lesions outside the probe and obtain the relationship between the probe and lesion when the ultrasound probe reaches the lumen of the bronchi. The use of a radial ultrasound probe helps "visualize" the lesion, greatly improves the accuracy of biopsy, and significantly improves the diagnostic yield of peripheral lung cancer.[6] However, it remains difficult to determine whether rpEBUS has obtained an adequate diagnostic specimen. ROSE has been applied in clinical practice to solve this problem.

ROSE has received increasing attention in the evaluation and preliminary diagnosis of respiratory disease lesions, especially peripheral lung lesions, and it has been shown to increase the diagnostic yields.[7] Our results showed that ROSE had a good diagnostic value in the diagnosis of PPLs. ROSE combined with rpEBUS can improve the quality of minimally invasive sampling, reduce unnecessary biopsies and punctures, reduce procedure times, and facilitate rapid diagnosis.

The characteristics of the lesion itself can influence the diagnosis by EBUS. Previous

Table 4. Effect of rpEBUS-GS-TBLB on diagnostic yield.

Variable	N	Diagnostic yield	χ^2	P-value
Size of lesion (mm)			6.760	0.014
8–20	46	28/46 (60.9%)		
20–30	60	50/60 (83.3%)		
Feature of PPL			5.469	0.025
Pure or mixed GGO	41	25/41 (61.0%)		
Solid nodule	65	53/65 (81.5%)		
Position of the probe			11.040	0.002
Within	66	58/66 (87.9%)		
Adjacent to	34	20/34 (58.8%)		
Invisible	6	1/6 (16.7%)		
Location of lesion			6.963	0.013
Upper	29	16/29 (55.2%)		
Middle or lingular	35	30/35 (85.7%)		
Lower	42	32/42 (76.2%)		

rpEBUS-GS-TBLB, radial probe endobronchial ultrasound transbronchial lung biopsy with guide sheath; PPL, peripheral pulmonary lesion; GGO, ground-glass opacity.

Table 5. Comparison of variables between ROSE-positive and ROSE-negative procedures.

Group	ROSE positive	ROSE negative	P-value
Biopsy number (n)	1.6 ± 0.9	2.8 ± 0.6	0.001
Procedure time (minutes)	20.5 ± 7.9	28.3 ± 7.6	0.005

ROSE, rapid on-site evaluation.

studies have indicated that lesion size affects the diagnostic yield of EBUS.[8,9] In this study, the diagnostic yield of PPLs ≥20 mm in diameter was significantly greater than that of PPLs <20 mm in diameter. This result suggested that EBUS combined with ROSE could improve the diagnostic yield of PPLs.

In this study, the diagnostic yields of each lobe were different, which is consistent with the low diagnostic yield reported for the upper lobes.[10] One possible reason for this is that the bending angle of the upper lobe is too large, making it difficult for the ultrasonic probe to reach the lesion site and obtain a satisfactory specimen, which in turn leads to a low diagnostic yield. In this study, under the guidance of ROSE, the brush path was adjusted according to the path determined by the probe to reach the position of the lesion and improve the diagnostic yield.

The relationship between the rpEBUS probe position and focus also affects the diagnostic yield of rpEBUS-TBLB.[11–15] Uchimura et al.[16] reported that the diagnostic yield when the EBUS probe was within the lesion was as high as 90%, but that diagnostic yield may be reduced to 52% to 55% when the ultrasound probe was adjacent to the lesion. Our study showed similar results. Nodule features also affected the diagnostic yield: the yield of solid nodules was higher than that of ground-glass nodules.

When ROSE gave a definite diagnosis, number of biopsies and procedure time were decreased. Reducing the number of

biopsies can reduce the number of slides and the cost of pathological examination. Shortening the procedure time can reduce adverse physiological effects on patients.[17–19] When ROSE is positive, the subsequent biopsy can be stopped, which reduces the incidence of bleeding, pneumothorax, and chest pain.

At present, there is a critical lack of pulmonary or respiratory endoscopists who can interpret cytological smears. Therefore, ROSE is performed mostly by cytology experts. Because of the limited number of cytology experts and the associated economic cost (labor cost and salary), development of ROSE is difficult. In this study, ROSE was performed by cytologists and respiratory physicians who had received professional and stringent pathology and cytology training.

This study has several limitations. First, this was a single-center, retrospective study. Second, these procedures were performed without the use of X-ray fluoroscopy. Finally, we used Diff-Quick staining instead of modified Gill-Shorr staining or modified Papanicolaou staining to prepare slides. Diff-Quick is a rapid method but less accurate than the other methods. We plan to conduct a prospective, randomized, multicenter study to further confirm the value of ROSE during rpEBUS. At the same time, we need to explore the best staining method for ROSE in future studies.

In summary, rpEBUS combined with ROSE is a safe and feasible method for diagnosis of PPLs.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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