

## Original Research Article

# Diagnostic value of sheath-guided frozen biopsy technique for drug-induced interstitial lung disease

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### Abstract

**Purpose:** To investigate the diagnostic value of sheath-guided frozen biopsy technique for interstitial lung disease (ILD).

**Methods:** In this retrospective study, 48 ILD patients of exclusive etiology admitted to Xi'an International Medical Center Hospital between October 1, 2021, and May 31, 2022, were randomly assigned to receive either endobronchial ultrasound with a guide sheath combined with transbronchial cryobiopsy TBCB (TBCB group), or endobronchial ultrasound with a guide sheath combined with transbronchial lung biopsy TBLB (TBLB group).

**Results:** The two groups were well-balanced in terms of baseline profiles ( $p = 0.94$ ). The mean size of tissue blocks was  $24.45 \pm 2.83 \text{ mm}^2$  in TBCB group and  $3.58 \pm 0.89 \text{ mm}^2$  in TBLB group. TBCB and TBLB groups presented with a diagnostic accuracy of 64.00 % (16/25) and 69.57 % (16/23), respectively, while chi-square test demonstrated no significant difference between the two groups ( $p = 0.683$ ). Chi-square test showed that there was significant difference in the incidence of complications ( $p = 0.036$ ) as well as the incidence of bleeding ( $p = 0.045$ ), but no significant difference in the incidence of pneumothorax between the two groups ( $p = 0.605$ ).

**Conclusion:** There are no significant differences in diagnostic sensitivity, specificity, and accuracy between endobronchial ultrasound sheath-guided frozen lung tissue biopsy and conventional clamped lung tissue biopsy for ILD, but the former has a lower incidence of complication than the latter. It is necessary to conduct a larger clinical study to further validate these findings.

**Keywords:** Sheath-guided, Frozen biopsy of lungs, Interstitial lung disease, Diagnostic value

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## INTRODUCTION

Interstitial lung disease (ILD) is a heterogeneous disease manifested by chronic inflammatory response or pulmonary fibrosis. Many drugs can cause ILDs, and ILDs related to molecularly targeted drugs have been frequently reported in recent years [1]. It involves the interstitial and

alveolar spaces of the lungs and is clinically characterized by progressively worsening dyspnea, restrictive ventilatory dysfunction with reduced diffusion function, and hypoxemia [1,2]. Pulmonary fibrosis is irreversible, and the progressive evolution of ILD thus poses a serious threat to the respiratory health and quality of life of patients [3]. The ILD progresses in either a

slow or aggressive manner, accompanied by a variety of autoimmune connective tissue diseases, which, if not effectively diagnosed will develop into diffuse pulmonary fibrosis and "honeycomb lung", leading to depletion of alveolar diffusion and eventual death due to respiratory failure [4,5]. Therefore, early diagnosis of ILD is particularly crucial.

Presently, imaging is the primary method for screening and diagnosis of ILD. The continuous evolution of computed tomography (CT) technology and the ever-increasing resolution of CT provides a more accurate diagnosis of ILD [6]. However, misdiagnoses for various reasons have been reported each year, thereby delaying the standardized treatment of ILD and further aggravating the condition [7]. The current clinical dilemma lies in timely and accurate diagnosis of interstitial lung disease.

A pathological biopsy is the gold standard for diagnosis of ILD, and this diagnosis and classification of ILD depend on lung histopathological biopsy. Samples of lung histopathological biopsy are obtained through transbronchial forceps biopsy (TBFB), percutaneous lung biopsy (PCLB), and surgical lung biopsy (SLB) [8-10], among which TBFB is widely used clinically due to its less invasiveness, rapidness, and high safety [11]. Nonetheless, these sampling methods cannot accurately locate the lesion, so multiple sampling is required. In addition, blind puncture sampling may damage large blood vessels and increase the risk of death due to blood loss [12]. Endobronchial ultrasound with a guide sheath combined with transbronchial lung biopsy (TBLB) is therefore developed to enhance the diagnostic accuracy of the current methods. In this technique, a bronchoscope is sent to the distal bronchial segment where the dense area of the lesion is shown on chest CT. A radial ultrasound probe is inserted into the guide sheath and sent along the biopsy orifice for ultrasound exploration to identify the best image of the lesion and then fix the sheath so that a biopsy can be performed [13]. This method increases diagnostic accuracy and reduces the risk of bleeding [14].

Endobronchial ultrasound with a guide sheath combined with transbronchial cryobiopsy (TBCB) is an upgrade from TBLB. It uses refrigerant to rapidly cool the tip of the cryoprobe to form ice crystals that adhere to lung tissue to maximize the integrity of the biopsy tissue and restore the original appearance of the lesion, thereby improving diagnostic accuracy. This study was performed to investigate the diagnostic value of

two sheath-guided frozen biopsy techniques for interstitial lung disease.

## METHODS

### Patients

In this retrospective study, 48 ILD patients of exclusive etiology admitted to Xi'an International Medical Center Hospital between October 1, 2021, and May 31, 2022, were randomly assigned to receive either endobronchial ultrasound with a guide sheath combined with transbronchial cryobiopsy (TBCB; TBCB group), or endobronchial ultrasound with a guide sheath combined with transbronchial lung biopsy (TBLB; TBLB group). This study was approved by the Ethics Committee of Xi'an International Medical Center Hospital (approval no. X20201001), and all procedures were conducted in accordance with the protocol of the Declaration of Helsinki [15].

### Inclusion criteria

Patients with imaging showing nodular, lamellar, honeycomb, cornular, and ground glass shadows of varying sizes in both lobes or more than 50 % of the lung fields, and whose diagnosis was undetermined after laboratory tests, imaging, and routine bronchoscopy were recruited.

### Exclusion criteria

Patients with contraindications to bronchoscopy beyond correction, imaging manifestations of infectious pneumonia, signs of lung neoplasm, clinical features inconsistent with a diagnosis of interstitial pneumonia, and histological evidence available to exclude a diagnosis of interstitial pneumonia, with acute myocardial infarction, active hemoptysis, platelet count  $< 60 \times 10^9/L$ , contraindications such as a malignant arrhythmia, unstable angina, severe cardiopulmonary insufficiency, hypertensive crisis, severe pulmonary hypertension, intracranial hypertension, acute cerebrovascular events, aortic coarctation, aortic retention, severe mental illness, coagulation abnormalities, and circulatory failure within 4 weeks before surgery, or who have not undergone routine investigations such as enhanced CT, coagulation function, blood routine, and blood biochemistry before surgery were excluded.

### Pre-operative preparation

Both groups received preoperative examinations, including blood, urine, and stool investigations, coagulation function, blood biochemistry, cardiac

enzyme profile, tumor markers, blood gas analysis, electrocardiogram, echocardiogram, pulmonary function, enhanced CT of lung, and infection screening. These values were used as the baseline for patient profiles. Other parameters recorded include gender, age, current medical history, past history, medication history, food-drug allergy history, family history, and personal history. Anticoagulants such as aspirin, clopidogrel, and warfarin were discontinued before surgery, and low-molecular heparin replacement therapy was administered for more than 7 days. The patients fasted 6 h before surgery.

### Operation steps

With the patient in a supine position, intravenous access was established before surgery, and intravenous anesthesia with remifentanyl and propofol was administered to maintain the BIS value in the range from 40 to 60. Trachea was intubated after anesthesia, and the pressure of the frozen air source was maintained at 50 - 60 bar. In any case of high resistance or violent airway reaction during intubation, an appropriate amount of lidocaine was administered for local anesthesia. During intubation, the vital signs of the patient were monitored, and 2 - 3 lesion sites indicated by preoperative CT imaging were selected as biopsy-targeted sites, and each site was biopsied 2 - 5 times. The ultrasound probe (Olympus, UM-S20-17S, Japan) and the guiding sheath were retrieved through the bronchoscopic biopsy orifice using a flexible bronchoscope (Flexible scope, Olympus, BT-IT260, Japan) through the tracheal intubation into the opening of the bronchial segment. The hypoechoic dense area was probed at the distal end of the bronchial segment where the biopsy target site was located to mark the probe. The bronchial subsegment and depth of entry were marked, the ultrasound probe was retrieved, and a 2.4 mm diameter cryoprobe (ERBE, Erbokryo CA, Germany) was used in the TBCB group to reach the same depth of the same bronchial subsegment along the guiding sheath. The bronchoscope with the probe was gently pulled out of the airway and thawed in saline immediately after 3 - 5 sec. The above operation was repeated 2 - 3 times, with a maximum of 3 biopsies per site from one patient. The TBLB group received biopsies using biopsy forceps along the guiding sheath into the same bronchial subsegment at the same depth. Ten tissue specimens were obtained for each site per patient.

It is recommended to have intraoperative supplies readily available, including iced normal

saline, 0.1% epinephrine, pituitrin, and thrombin. In the event of bleeding during the operation, continuous bronchoscope suction was employed, and the drugs were applied locally in the airway to control bleeding. If these measures were ineffective and bleeding persisted, further hemostatic measures such as a balloon or hemostatic gauze plugging were utilized. Selective bronchial artery embolization or surgical intervention may be necessary for hemostasis. Additionally, vigilance for pneumothorax was advised during the operation.

Postoperatively, the patients received oxygen therapy for 4 h. Blood pressure, oxygen saturation, electrocardiogram, and respiratory monitoring were carried out for 24 h daily. In case of bleeding, dyspnoea, pneumothorax, and other complications, symptomatic hemostasis, mask oxygenation, tracheal intubation, or tracheotomy were adopted as required. If intraoperative bleeding occurred, constant bronchoscopic aspiration was maintained while local application of the above-mentioned drugs in the airway was used to stop the bleeding. If bleeding persisted, a balloon or hemostatic gauze was used for hemostasis, and selective bronchial artery embolization or surgical hemostasis was adopted where necessary.

### Preservation and treatment of specimens

Specimens taken by TBCB were immediately thawed in physiological saline, and the measurement of tissue size (A) is shown in Eq 1.

$$A (\text{mm}^2) = (\text{LD} \times \text{SD}) \dots\dots\dots (1)$$

where LD is long diameter and SD is short diameter of the specimen determined within 1 min after natural detachment of the tissue block.

The sample was then quickly fixed in 4 % formaldehyde solution and sent to the pathology department for paraffin embedding and staining within 6 h after measurement. Serial sections were examined under a light microscope or electron microscope and routinely subjected to periodate-chev staining and antacid staining, and immunohistochemical staining was performed at the discretion of the pathologists. Specimens taken by TBLB were fixed in 4 % formaldehyde solution immediately after completion of their size measurement, followed by the same treatment as above.

### Pathology and etiology diagnosis

Histopathological diagnoses of TBCB and TBLB were completed by two physicians with at least

10 years of experience in the Department of Pathology of Xi'an International Medical Center Hospital. The pathological diagnostic criteria mainly referred to the 2013 American Thoracic Society/European Respiratory Society classification criteria for idiopathic interstitial pneumonia and the Chinese expert consensus on the diagnosis and treatment of idiopathic pulmonary fibrosis [16]. For patients lacking characteristic pathological manifestations and definitive pathological diagnosis, the final disease typing was arrived at after multidisciplinary discussions between the Department of Respiratory Medicine and the Department of Pathology in conjunction with other multidisciplinary departments.

### Diagnostic criteria for complications

Postoperative complications following TBCB and TBLB primarily involved hemorrhage and pneumothorax, with their respective grading and evaluation criteria outlined in Table 1 and Table 2. Additional complications include subcutaneous emphysema, mediastinal emphysema, infection, airway laceration, and a vocal cord or laryngeal injury.

**Table 1:** Evaluation criteria of hemorrhage grading

Hemorrhage grading	Definition
Grade 0	No bleeding.
Grade 1	No continuous suction is required and bleeding stops spontaneously.
Grade 2	Bronchial segments that required bronchoscopic obstruction of the biopsy by bronchoscopy are hemostatically treated with local epinephrine or ice saline.
Grade 3	Required placement of a bronchial obstruction balloon or catheter, surgical intervention, and use of systemic coagulants.
Grade 4	It leads to transfusion, asphyxiation, intubation, CPR, or death, requiring admission to the intensive care unit.

**Table 2:** Evaluation criteria for pneumothorax classification

Pneumothorax classification	Definition
A small amount of pneumothorax	Pulmonary compression $\leq$ 30%.
Medium volume pneumothorax	30% < pulmonary compression $\leq$ 50%
Massive pneumothorax	Pulmonary compression $\geq$ 50%

To diagnose subcutaneous emphysema and mediastinal emphysema, the skin of the neck and lungs are examined by percussion or

auscultation after the biopsy. If there is a twisting sensation on palpation of the skin of the neck and chest, or if breath sounds on the biopsy sites are significantly reduced or absent, C-arm or ultrasonography should be utilized to evaluate the occurrence of these complications.

The patients were closely monitored for temperature and respiratory symptoms post-operatively. In the event of persistent postoperative fever, worsening respiratory symptoms, and a marked increase in peripheral blood leucocyte count, pathogenic investigations were performed to determine the presence of postoperative infection.

The patients were monitored postoperatively for discomforts such as persistent hemoptysis, pharyngeal pain, hoarseness, or voicelessness.

### Statistical analysis

SPSS 26.0 statistical software was used for data analysis. Measurement data are expressed as mean  $\pm$  standard deviation (SD) and counting data are expressed as n (%). Inter-group comparison of measurement data was determined by *t*-test, and chi-square test was performed for counting data. Statistically significant difference was indicated by  $p \leq 0.05$ .

## RESULTS

### Clinical baseline data

A total of 48 patients with interstitial pneumonia of unknown etiology were included, including 28 males and 20 females, with a mean age of  $56.88 \pm 8.28$  years. Twenty-five patients were recruited in TBCB group, including 15 males and 10 females, with a mean age of  $54.92 \pm 8.42$  years old. In the TBLB group, there were 23 cases, involving 13 males and 10 females, with a mean age of  $59.0 \pm 7.75$  years. The two groups were balanced in terms of baseline patient profiles, as shown in Table 3.

### Satisfactory sampling rate

Satisfactory sampling refers to a smooth procedure with no serious complications and a definitive pathological diagnosis as evidenced by the presence of alveolar epithelial cells and Langerhans cells in the sections. The failed sampling was defined as a serious intraoperative complication that necessitates termination of the procedure, or the failure to obtain pathological tissue in response to multiple attempts by the physician and for various other reasons.

**Table 3:** Inter-group comparison of general data

Parameter	TBCB group	TBLB group	X <sup>2</sup> /t	P-value
Age (years)	54.92 ± 8.42	59.00 ± 7.75	1.742	0.09
Gender (Male)	60.0% (15/25)	56.5% (13/23)	0.06	0.81
Smoking history	52.0% (13/25)	47.8% (11/23)	0.08	0.77
Pre-existing underlying disease	36.0% (9/25)	30.4% (7/23)	0.17	0.68
Coughing	80.0% (20/25)	82.6% (19/23)	0.05	0.82
Sputum	68.0% (17/25)	56.5% (13/23)	0.67	0.41
Shortness of breath	40.0% (10/25)	39.1% (9/23)	0.00	0.95
Fever	28.0% (7/25)	43.5% (10/23)	1.26	0.26
D-dimer abnormalities	40.0% (10/25)	39.1% (9/23)	0.00	0.95
CEA abnormalities	44.0% (11/25)	43.5% (10/23)	0.00	0.971
NSE abnormalities	40.0% (10/25)	47.8% (11/23)	0.30	0.59
Abnormal blood gas analysis	36.0% (9/25)	47.8% (11/23)	0.69	0.41
Lung dysfunction	80.0% (20/25)	78.2% (18/23)	0.02	0.88
Abnormal tumor markers	36.0% (9/25)	47.8% (11/23)	0.69	0.41
Abnormal liver function	28.0% (7/25)	39.1% (9/23)	0.67	0.41
Abnormal cardiac enzyme profile	20.0% (5/25)	30.4% (7/23)	0.70	0.40

the TBLB group recorded 21 successful biopsies and 2 failures attributable to intraoperative respiratory failure and unavailability of a sufficient number of pathological tissues. The operation failure rate was 4.0 % in the TBCB group and 8.7 % in the TBLB group, with no statistically significant difference between the two groups ( $p = 0.94$ ), as shown in Table 4.

**Table 4:** Comparison of sampling failure rate between TBCB group and TBLB group

Group	Satisfied with sampling	Sampling failure	Chi-squared	P-value
TBCB	24	1	0.01	0.94
TBLB	21	2		

#### Size of tissue blocks

The mean size of tissue blocks obtained in TBCB group was  $24.45 \pm 2.83 \text{ mm}^2$  and  $3.58 \pm 0.89 \text{ mm}^2$  in TBLB group. An independent samples  $t$ -test for tissue size showed a statistically significant difference between the results of the two groups ( $t = 34.99$ ,  $p = 0.000$ ).

#### Pathology and follow-up results following biopsy

In the TBCB group, 24 patients were successfully sampled, of which 17 were pathologically diagnosed with ILD, 4 with chronic inflammation, 2 with adenocarcinoma, and 1 with squamous cell carcinoma. In the TBLB group, 21 cases were collected successfully, amongst which 18 cases were pathologically diagnosed as ILD, and 3 cases were diagnosed with chronic inflammation. During follow-up, 16 of the 17 patients in the TBCB group were diagnosed with ILD, and one was confirmed with

adenocarcinoma. In TBLB group, 16 of the 18 patients were diagnosed with ILD, and the remaining 2 were determined to be adenocarcinoma at follow-up. One of the 3 patients with chronic inflammatory disease was identified, and 2 others were confirmed as ILD at follow-up, as shown in Table 5.

**Table 5:** Pathological diagnosis and follow-up results of TBCB and TBLB groups

Group	Diagnosis	Standard	
		Diagnosed with ILD (n)	Diagnosed with non-ILD (n)
TBCB	Diagnosed with ILD	16	1
	Diagnosed with non-ILD	1	6
TBLB	Diagnosed with ILD	16	2
	Diagnosed with non-ILD	2	1

#### Sensitivity, specificity, and accuracy

As shown in Table 5, the diagnostic sensitivity in TBCB group and TBLB group for ILD was 94.12 % (16/17) and 88.89 % (16/18) ( $p = 1.00$ ). Concerning the diagnostic specificity, TBCB and TBLB group were 85.71 % (6/7) and 33.33 % (1/3) ( $p = 0.183$ ). The false-negative rate and false-positive rate of the diagnosis of ILD in TBCB group were 5.88 % (1/17) and 14.29 % (1/7), and corresponding values of TBLB group were 11.11 % (2/18) and 66.67 % (2/3), respectively. The TBCB and TBLB group presented with a diagnostic accuracy of ILD of 64.00 % (16/25) and 69.57 % (16/23), respectively, and the chi-square test

**Table 6:** Comparison of sensitivity, specificity, and accuracy between TBCB group and TBLB group

Parameter	TBCB group (n = 25)	TBLB group (n = 23)	P-value
Sensitivity	94.12% (16/17)	88.89% (16/18)	1.00
Specificity	85.71% (6/7)	33.33% (1/3)	0.183
Accuracy	64.00% (16/25)	69.57% (16/23)	0.683
False negative rate	5.88% (1/17)	11.11% (2/18)	
False positive rate	14.29% (1/7)	66.67% (2/3)	

**Table 7:** Complication rates in TBCB group compared with those in TBLB group (n=25)

Parameter	TBCB group	TBLB group	P-value
Incidence of complications	44.0% (11/25)	73.9% (17/23)	0.036
Hemorrhage	32.0% (8/25)	60.9% (14/23)	0.045
Pneumothorax	12.0% (3/25)	21.7% (5/23)	0.605
Other	0.0%	0.0%	

demonstrated no statistically significant difference between the two groups ( $p = 0.683$ ), and the above results were summarized in Table 6.

### Complication rates

The complication rate in the TBCB group was 44.0 % (12/25), including 32.0 % (8/25) for grade 1 bleeding and 12.0 % (3/25) for a small amount of pneumothorax. Similarly, the complication rate in the TBLB group was 73.9 % (17/23), including 60.9 % for bleeding (14/23), with 3 cases of grade 2 bleeding and 11 cases of grade 1 bleeding. The incidence of pneumothorax was 21.7 % (5/23), with 1 case of moderate pneumothorax and 4 cases of small amount of pneumothorax. The chi-square test exhibited a statistically significant difference in the incidence of complications ( $P = 0.036$ ) as well as the incidence of bleeding ( $p = 0.045$ ), but no statistically significant difference in the incidence of pneumothorax between the two groups ( $p = 0.605$ ), as shown in Table 8.

## DISCUSSION

Currently, endobronchial ultrasound-guided lung tissue cryobiopsies have been extensively employed in clinical practice for the diagnosis of ILD with undetermined causes [14]. However, few studies have been conducted on the diagnostic value of TBCB, with a paucity of evaluation of its efficacy and safety [17]. An earlier study has reported an overall diagnostic rate of 80.6 % and the incidence of pneumothorax to be 11.1 % [17]. Some researchers have used EBUS-TBCB to conduct biopsies on patients and reported higher

diagnostic accuracy, shorter procedure time, and lower complication rates [18]. In the present study, 25 patients received TBCB, and 16 were diagnosed with ILD with a diagnostic sensitivity of 94.12 % (16/17), specificity of 85.71 % (6/7), and accuracy of 64.00 % (16/25). The remaining 23 patients received TBLB and 16 of them were diagnosed, with a diagnostic sensitivity of 88.89 % for ILD (16/18), specificity of 33.33 % (1/3), and accuracy of 69.57 % (16/23). This result is however inconsistent with some other earlier findings [18]. Nevertheless, in the present study, TBCB group had a lower complication rate ( $P = 0.036$ ) and a lower risk of bleeding ( $P = 0.045$ ) than TBLB group, which was consistent with results of a previous study [18]. The underlying reasons may be in the operator's proficiency during the operation, patients' cooperation, and sample size. In the present study, the sample tissues collected by TBCB were larger in size than those by TBLB, which rendered more comprehensive information on clinical diagnosis for pathologists. Moreover, TBCB group exhibited a lower complication incidence than TBLB group, suggesting that it has a higher safety profile.

### Limitations of this study

The small sample size was a major limitation of this study. Yet, this study was a pilot clinical study. Future studies will be required to investigate larger sample sizes and verify the treatment effects among the 3 groups. Second, due to the design characteristics, causality was not pursued, and a prospective and controlled trial in a larger sample will be needed to strengthen the hypothesis.

## CONCLUSION

TBCB and TBLB are comparable in terms of sampling satisfaction, but the former provides a larger sample area and comprehensive microscopical data than the latter one. In contrast, TBCB has no significant advantage over TBLB in terms of diagnostic sensitivity, specificity, and accuracy for ILD. However, TBCB is associated with a relatively lower incidence of complications and bleeding risk. There is a need to conduct a larger clinical study to validate the diagnostic value of TBCB for ILD.

## DECLARATIONS

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None provided.

### Funding

None provided.

### Ethical approval

This study was approved by the Ethics Committee of Xi'an International Medical Center Hospital, China (approval no. X20201001),

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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