

Diagnosis of Diffuse Parenchymal Lung Disease Using Transbronchial Cryobiopsy in an Ambulatory Setting

Dear Editor,

Diffuse parenchymal lung disease (DPLD) is encountered not only in pulmonary medicine as idiopathic interstitial pneumonias or interstitial lung disease (ILD), but also in transplant medicine, infectious disease, connective tissue diseases and drug-induced ILDs.¹ The incidence of DPLD in Singapore is currently unknown but its prevalence appears to be increasing worldwide. For example, the incidence of idiopathic pulmonary fibrosis in North America and Europe is 3 to 9 cases per 100,000 person-years and in South America and East Asia, it is reported as <4 cases per 100,000 person-years.² Treatment of DPLD is different ranging from steroids to antimicrobials to withdrawal of an implicated drug. Therefore, it is imperative to establish an accurate diagnosis.

Data show that samples from transbronchial biopsy via flexible bronchoscopy may only provide a confirmed diagnosis in 20% to 30% of patients with ILD, even when combined with clinical and high resolution computed tomography (CT) input.³ Biopsy samples are limited in size³ and subject to crush artefact⁴ from both the closure of forceps, as well as withdrawal through the narrow working channel of the scope. Surgical lung biopsies have a diagnostic yield of 93% to 98% but mortality ranges from 1.7% to 2.7%.⁴⁻⁶ In addition, mortality is significantly higher in non-elective biopsies (16%) and risks are related to age and co-morbidities.⁵ These factors have driven research in obtaining high quality lung biopsy specimens in a safer manner.

Bronchoscopic cryobiopsies can obtain larger specimens⁷ and meta-analyses data suggest that the diagnostic yield for DPLD is 83.7%.⁶ The Joule-Thomson effect of a rapidly expanding gas (carbon dioxide) is utilised to reduce the temperature of the cryoprobe tip to -79°C. This causes cryo-adherence to tissue which then allows large biopsies to be taken. Because the scope and the cryoprobe are removed together in order to recover the sample without passing through the working channel of the bronchoscope, there is the added advantage of avoiding crush artifact.⁸ Cryobiopsies have a procedure-related mortality of 0.3% and median hospitalisation is 2.6 days.⁴ There is also evidence to show that bronchoscopic cryobiopsies are able to increase the diagnostic confidence in the multidisciplinary diagnosis of idiopathic pulmonary fibrosis.⁹

However, there is limited data of performing transbronchial cryobiopsies in an ambulatory setting under moderate sedation, without the need for endotracheal intubation, general anaesthesia or endobronchial blockers. It is envisaged that the number of specimens that can be obtained may be smaller and the diagnostic yield of such a compromise is also unknown. The attraction of this ambulatory approach goes beyond reduced costs. It may make cryobiopsies widely available because minimal additional equipment and personnel will be needed compared to routine flexible bronchoscopy. The aim of this pilot study is to evaluate the safety and diagnostic yield of transbronchial cryobiopsy for DPLD in an ambulatory setting in Singapore.

Materials and Methods

Prospectively collected data from a bronchoscopy database included all patients from Singapore General Hospital who underwent transbronchial cryobiopsy for DPLD. Data from July 2015 to November 2017 were analysed in this study. Inclusion criteria were age 18 to 80, and presence of diffuse lung disease on CT that was consistent with DPLD. Exclusion criteria included contraindication to bronchoscopy such as haemodynamic instability, respiratory failure, uncorrected coagulopathy, as well as suspected pregnancy or focal lung disease on chest radiology. Patients who had CT findings that met all criteria for a radiological diagnosis of usual interstitial pneumonia were excluded as well and managed accordingly.¹⁰ Institutional review board approval (no.: 2011/350/C) was obtained and ClinicalTrials.gov identifier is NCT01374542.

A positive diagnostic result was defined as either a confirmed histopathological or microbiological diagnosis that was both compatible with the clinical picture and did not require further diagnostic testing. Patients with non-diagnostic biopsies were treated empirically based on the clinical diagnosis or followed up with radiological surveillance for at least 6 months. None of the patients were subject to alternative biopsy procedures. Bronchoscopic complications such as pneumothorax, bleeding, hypoxia, and hypotension were recorded down whenever they were present and were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.¹¹

Cryobiopsies were performed with a flexible cryoprobe (Erbecryo, Erbe Elektromedizin GmbH, Tübingen, Germany). This probe has an outer diameter of 1.9 mm and a length of 900 mm. All procedures were performed under moderate sedation using a combination of fentanyl and midazolam. Bronchoscopic intubation was done orally instead of nasally. This allowed for extubation once the specimen was obtained and subsequent re-intubation. No endotracheal tube or rigid bronchoscope was used. Bronchoalveolar lavage was first performed in the targeted subsegment of the lung before cryobiopsy was performed. The cryobiopsy freeze cycle was set at 4 seconds and after obtaining the biopsy, the tip of the cryoprobe was placed in a container of normal saline to facilitate rapid thawing. The specimen was then recovered from the container of saline and placed in formaldehyde before being sent to the histopathology laboratory. Fluoroscopy was utilised for all cryobiopsies and the probe was activated <10 mm from the pleural margin.

Results

Transbronchial cryobiopsy was performed in 22 consecutive patients presenting with DPLD. Demographic and procedure details are shown in Table 1. Cryobiopsy sample size was 5.1 ± 1.7 mm in diameter and alveolar tissue was identified in all samples. The mean alveolar surface area per sample was 8.5 ± 5.6 mm². Large airways were present in 15/22 (68%), small airways in 100% and large vessels in 19/22 (86%). It was possible to do histopathological stains on all specimens and the cryobiopsy technique does not appear to impede or distort histological investigations.

A positive diagnostic result from the bronchoscopy was obtained in 16/22 (73%) patients (Table 2). Cryobiopsy histology was diagnostic in 10/22 (45%) and microbiology was positive from bronchoalveolar lavage in 6/22 (27%). Examples of definitive cryobiopsy results are shown in Figure 1. The remaining 6 patients without a diagnosis were discussed at multidisciplinary meetings before a diagnosis was assigned. In our follow-up, 1 patient with post-inflammatory scar and 3 patients with bacterial pneumonia were considered to be true negatives.

Complications were documented in 7/22 cases (32%). Six patients experienced moderate endobronchial bleeding immediately postbiopsy and were managed by local therapy such as ice cold saline and tamponade of the endobronchial segment with the flexible bronchoscope. One patient required 500 cc of intravenous normal saline for transient hypotension. None of the patients required endobronchial blocker placement. One other patient developed hypoxaemia that needed face mask and bagging for <1 min. This was attributed to the sedation given. No ventilator support was required postprocedure. All patients had screening

Table 1. Demographics and Procedure Details

	n (%)
Total number of patients	22
Female gender	11 (50)
Median age in years (range)	58.5 (26 – 77)
Chinese ethnicity	12 (55)
Never smokers	12 (55)
ASA scoring	
ASA 1	5 (23)
ASA 2	10 (45)
ASA 3	7 (32)
CT findings*	
Ground glass opacification	8 (36)
Nodular opacities	7 (32)
Consolidative infiltrates	10 (45)
Mean duration of bronchoscopy in minutes	20.7 ± 4.7
Sedation	
Median midazolam dose in mg (range)	4 (2 – 5)
Median fentanyl in mcg (range)	100 (50 – 100)
Median number of cryobiopsy samples per patient (range)	2 (1 – 4)
Mean diameter of biopsy sample in mm	5.1 ± 1.7
Biopsy site	
One bronchopulmonary segment	15 (68)
Two bronchopulmonary segments	7 (32)
Complications†	
Pneumothorax	0
Bleeding grade 2	6 (27)
Hypoxaemia grade 3	1 (5)

ASA: American Society of Anesthesiologists; CT: Computed tomography
*With overlaps to account for multiple findings.

†Descriptions found at: National Cancer Institute NCI, US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. 2009. Available at: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Accessed on 6 September 2017.

fluoroscopy at the end of the bronchoscopy and then a check chest radiograph. No pneumothorax was detected. None of the patients required escalation of care and no one was readmitted following the bronchoscopy.

Discussion

Data from our pilot study showed that transbronchial cryobiopsy can be performed safely in patients presenting with DPLD in an ambulatory setting, under moderate sedation and without the deployment of an endobronchial blocker. No rigid bronchoscope or endotracheal tube was necessary. The only other previous report in the literature to describe this technique necessitated 2 bronchoscopes, 2

Table 2. Clinical-Radiological-Pathological Correlation and Final Diagnosis of All Patients

Patient Age/Gender/Ethnicity	CT Findings			Cryobiopsy Histology	Bronchoalveolar Lavage Microbiology	Diagnosis on Clinical and Radiological Follow-up
	GGO	Nodules	Consolidation			
41/F/I	×	✓	×	Hypersensitivity pneumonitis	Non-diagnostic	Hypersensitivity Pneumonitis
73/F/C	✓	×	×	Drug-induced ILD	Non-diagnostic	Drug-induced ILD
70/M/E	✓	×	×	Drug-induced ILD	Non-diagnostic	Drug-induced ILD
41/F/I	✓	×	×	Drug-induced ILD	Non-diagnostic	Drug-induced ILD
57/M/C	✓	×	✓	Drug-induced ILD	Non-diagnostic	Drug-induced ILD
67/M/C	✓	×	×	Organising pneumonia	Non-diagnostic	Cryptogenic organising pneumonia
69/F/C	×	×	✓	Organising pneumonia	Non-diagnostic	Cryptogenic organising pneumonia
72/F/C	×	×	✓	Organising pneumonia	Non-diagnostic	Cryptogenic organising pneumonia
60/F/C	✓	✓	×	Chronic interstitial pneumonia (lymphocytic interstitial pneumonia like)	Non-diagnostic	Lymphocytic interstitial pneumonia
52/M/C	×	✓	×	Foreign body reaction	Non-diagnostic	Foreign body reaction
53/F/C	×	×	✓	Non-diagnostic	<i>Klebsiella pneumonia</i>	<i>Klebsiella pneumonia</i>
54/M/O	×	×	✓	Non-diagnostic	Influenza A	Influenza A pneumonia
77/M/M	×	×	✓	Non-diagnostic	<i>Klebsiella pneumonia</i>	<i>Klebsiella pneumonia</i>
26/F/O	×	✓	×	Non-diagnostic	Tuberculosis	Tuberculosis
75/M/C	×	✓	×	Non-diagnostic	Tuberculosis	Tuberculosis
74/F/O	×	×	✓	Non-diagnostic	Non-tuberculous mycobacteria	Non-tuberculous mycobacteria
51/M/O	✓	×	×	Non-diagnostic	Non-diagnostic	Desquamative interstitial pneumonia
54/F/C	✓	×	✓	Non-diagnostic	Non-diagnostic	Postinflammatory scar
62/M/C	×	×	✓	Non-diagnostic	Non-diagnostic	Bacterial pneumonia
34/M/I	×	×	✓	Non-diagnostic	Non-diagnostic	Bacterial pneumonia
67/F/C	×	✓	×	Non-diagnostic	Non-diagnostic	Bacterial pneumonia
52/M/I	×	✓	×	Non-diagnostic	Non-diagnostic	Non-diagnostic

C: Chinese; E: Eurasian; F: Female; GGO: Ground glass opacity; I: Indian; ILD: Interstitial lung disease; M: Male; M: Malay; O: Others

processors and 2 interventional pulmonologists.¹² Not only have we shown that this additional logistics is not necessary, but it is also possible to make a confirmed diagnosis in 73% of patients when microbiological evaluation from bronchoalveolar lavage was added to the histological analysis of cryobiopsy.

Conventional wisdom suggests that the larger samples from cryobiopsies result in substantial postbiopsy bleeding¹³ and necessitate the use of rigid bronchoscopy and pre-emptive endobronchial blocker deployment.^{14,15} Two meta-analyses showed that the risk of moderate bleeding was 12% (95% CI, 2.0% to 25%) and 4.9% (95% CI, 2.2% to 10.7%), respectively.^{4,6} Furthermore, it is thought to be easier to control bleeding when the patient is under deep

sedation or general anaesthesia compared to moderate sedation, especially when the patient starts coughing. Our data has challenged all these assertions.

The bleeding risk was mitigated in our study by limiting cryofreeze duration to 4 seconds in our protocol as opposed to 5 seconds or longer in other studies.⁴ In addition, fewer biopsies were obtained in our study with an average of 2 samples per patient. Moreover, in 68% of patients, only a single bronchopulmonary segment was sampled despite the presence of diffuse radiological infiltrates. Regardless of these limitations, the mean biopsy diameter met the 5 mm target that has been recommended for acceptable cryobiopsies.⁸ Other criteria require at least 1 fragment of alveolated lung parenchyma to classify the biopsy as adequate.¹⁵ All our samples met this adequacy criteria as well.

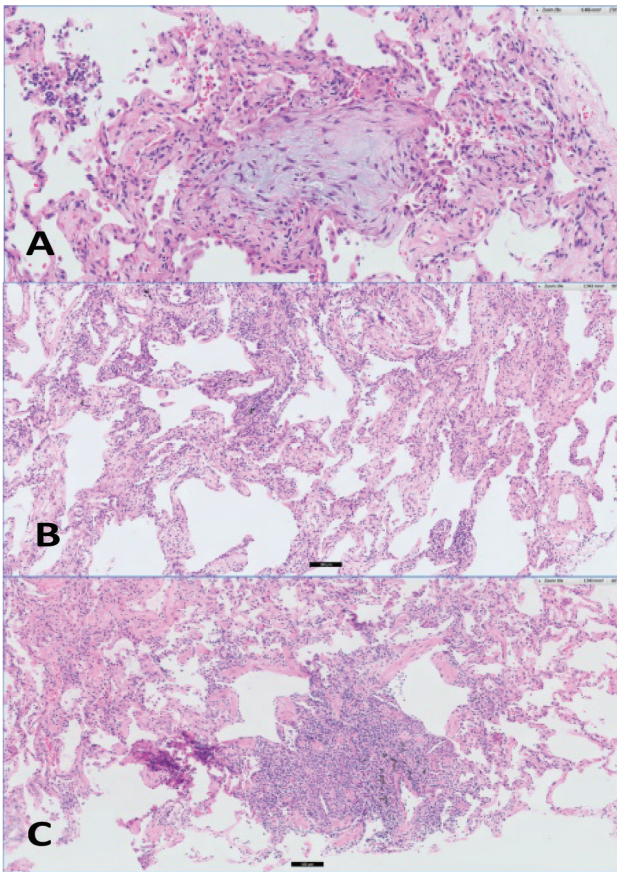


Fig. 1. Representative cryobiopsies providing definitive diagnoses after clinical radiological correlation: A) Organising pneumonia x20; B) Lymphocytic interstitial pneumonia x10; and C) Hypersensitivity pneumonitis x10.

There is the possible criticism that cryobiopsy alone contributed to a diagnostic yield of only 45% (10/22) in our study. However, on further analysis of the remaining 12/22 patients, 1 patient with desquamative interstitial pneumonia, 3 patients with mycobacterial disease and 1 further patient with no confirmed final diagnosis were truly non-diagnostic. In the remaining 6 cases of slowly resolving pneumonia and 1 case of postinflammatory scar, there were no pathognomonic histological features and hence non-specific inflammatory changes were correctly identified (7/22). Hence, among cases in which a diagnosis was actually possible on histology, the cryobiopsy samples were successful in 10/15 (67%). This is concordant with analysis of current data on diagnostic yield that ranges from 50% to >90%.⁴ Given the confidence gained from performing transbronchial cryobiopsies in this study, future attempts with this procedure should sample multiple bronchopulmonary segments because of data showing increased yield if samples are obtained from 2 segments within the same lobe rather than the same segment.¹³

The risk of pneumothorax from transbronchial cryobiopsy is 6% (95% CI, 2% to 11%) and 9.5% (95% CI, 5.9% to

14.9%) according to 2 recent meta-analyses.^{4,6} The limited number and size of biopsies are likely to have contributed to the lower risk in our pilot study. The limitations of this study is the small sample size and the fact that all procedures were carried out by only 1 endoscopist (DA) or read by just 1 histopathologist (AT). However, this can also be a strength because of standardisation of procedures. In addition, there were limited numbers of patients with fibrotic lung disease such as usual interstitial pneumonia or non-specific interstitial pneumonia. This may be reflective of a general respiratory practice with a predominance of pulmonary infections and drug-induced pneumonitis.¹⁶

Conclusion

Transbronchial cryobiopsies have become established in the evaluation of DPLD. As bronchoscopists, our commitment to patients is to perform the procedure such that diagnostic yield is maximised and complications are minimised while making the technology as widely available as possible. This pilot study has showed that transbronchial cryobiopsies can be carried out safely in an ambulatory setting under moderate sedation. With a shorter freeze time and fewer samples, a reasonable diagnostic rate is still possible. There is drive towards standardisation of the transbronchial cryobiopsy procedure internationally. However, before any guidelines are determined, the true range of options available should be evaluated and this includes performing transbronchial cryobiopsy under moderate sedation without endotracheal intubation, general anaesthesia or endobronchial blockers.

REFERENCES

1. American Thoracic Society, European Respiratory Society. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. *Am J Respir Crit Care Med* 2002;165:277-304.
2. Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. *Eur Respir J* 2015;46:795-806.
3. Sheth JS, Belperio JA, Fishbein MC, Kazerooni EA, Lagstein A, Murray S, et al. Utility of transbronchial vs surgical lung biopsy in the diagnosis of suspected fibrotic interstitial lung disease. *Chest* 2017;151:389-99.
4. Ravaglia C, Bonifazi M, Wells AU, Tomassetti S, Gurioli C, Piciucchi S, et al. Safety and diagnostic yield of transbronchial lung cryobiopsy in diffuse parenchymal lung diseases: a comparative study versus video-assisted thoracoscopic lung biopsy and a systematic review of the literature. *Respiration* 2016;91:215-27.

5. Hutchinson JP, Fogarty AW, McKeever TM, Hubbard RB. In-hospital mortality after surgical lung biopsy for interstitial lung disease in the United States. 2000 to 2011. *Am J Respir Crit Care Med* 2016;193:1161-7.
6. Ifthikhar IH, Alghothani L, Sardi A, Berkowitz D, Musani AI. Transbronchial lung cryobiopsy and video-assisted thoracoscopic lung biopsy in the diagnosis of diffuse parenchymal lung disease. A meta-analysis of diagnostic test accuracy. *Ann Am Thorac Soc* 2017;14:1197-211.
7. Griff S, Ammenwerth W, Schonfeld N, Bauer TT, Mairinger T, Blum TG, et al. Morphometrical analysis of transbronchial cryobiopsies. *Diagn Pathol* 2011;6:53.
8. Colby TV, Tomassetti S, Cavazza A, Dubini A, Poletti V. Transbronchial cryobiopsy in diffuse lung disease: update for the pathologist. *Arch Pathol Lab Med* 2017;141:891-900.
9. Tomassetti S, Wells AU, Costabel U, Cavazza A, Colby TV, Rossi G, et al. Bronchoscopic lung cryobiopsy increases diagnostic confidence in the multidisciplinary diagnosis of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2016;193:745-52.
10. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med* 2011;183:788-824.
11. National Cancer Institute NCI, US Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. 2009. Available at: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Accessed on 6 September 2017.
12. Bango-Alvarez A, Ariza-Prota M, Torres-Rivas H, Fernandez-Fernandez L, Prieto A, Sanchez I, et al. Transbronchial cryobiopsy in interstitial lung disease: experience in 106 cases – how to do it. *ERJ Open Res* 2017;3: pii 00148-2016.
13. Ravaglia C, Wells AU, Tomassetti S, Dubini A, Cavazza A, Piciocchi S, et al. Transbronchial lung cryobiopsy in diffuse parenchymal lung disease: comparison between biopsy from 1 segment and biopsy from 2 segments – diagnostic yield and complications. *Respiration* 2017;93:285-92.
14. Yarmus L, Akulian J, Gilbert C, Illei P, Shah P, Merlo C, et al. Cryoprobe transbronchial lung biopsy in patients after lung transplantation: a pilot safety study. *Chest* 2013;143:621-6.
15. Casoni GL, Tomassetti S, Cavazza A, Colby TV, Dubini A, Ryu JH, et al. Transbronchial lung cryobiopsy in the diagnosis of fibrotic interstitial lung diseases. *PLoS One* 2014;9:e86716.
16. Low SY, Koh MS, Ong TH, Phua GC, Anantham D. Use of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) in the diagnosis of granulomatous mediastinal lymphadenopathy. *Ann Acad Med Singapore* 2014;43:250-4.

Jane JX Lim, ¹*MBBS*, Angela Takano, ²*MBBS, AMBD*,
 Devanand Anantham, ³*MBBS, MRCP (UK), FAMS*

¹Yong Loo Lin School of Medicine, National University of Singapore, Singapore

²Department of Pathology, Singapore General Hospital, Singapore

³Department of Respiratory and Critical Care Medicine, Singapore General Hospital, Singapore

Address for Correspondence: Asst/Prof Devanand Anantham, Department of Respiratory and Critical Care Medicine, Academia, Level 3, Singapore General Hospital, 20 College Road, Singapore 169856.
 Email: anantham.devanand@singhealth.com.sg