CHEST

Original Research

INTERVENTIONAL PULMONOLOGY

Cryoprobe Transbronchial Lung Biopsy in Patients After Lung Transplantation

A Pilot Safety Study

Lonny Yarmus, DO, FCCP; Jason Akulian, MD; Christopher Gilbert, DO; Peter Illei, MD; Pali Shah, MD; Christian Merlo, MD; Jon Orens, MD, FCCP; and David Feller-Kopman, MD, FCCP

Background: **Transbronchial biopsies using standard forceps (FTBBxs) are often limited by crush artifact and their small size. To date, there have been no studies aimed at assessing the safety and efficacy of cryoprobe biopsies (CPBxs) in the population of patients who have undergone lung transplants. We present the safety profile and biopsy results from the first 21 procedures in a pilot study comparing CPBx to FTBBx in patients after lung transplantation.**

Methods: **Patients who had undergone lung transplant and who were scheduled for bronchoscopy were sequentially enrolled between November 2011 and September 2012. Inclusion criteria included age > 18 years and bilateral, orthotopic lung transplant. Exclusion criteria were coagulopathy, $FEV_1 < 0.8$ L, diffuse bullous disease, hemodynamic instability, and severe hypoxemia ($Pao_2 < 55$ mm Hg or $Spo_2 < 92\%$ on room air). Twenty-one procedures were performed, 10 using rigid bronchoscopy followed by 11 via flexible bronchoscopy. Patients were monitored for complications including pneumothorax, hemodynamic instability, and/or respiratory distress. Bleeding was categorized on an adapted grading system.**

Results: **Twenty-one procedures in 17 patients (median age: 52 years; 12 male patients) were performed. Specimen area and percent open alveoli were significantly greater using CPBx compared with FTBBx ($P < .05$). No clinically significant procedural complications occurred and all patients were discharged the day of the procedure.**

Conclusions: **The use of the cryoprobe is a safe, alternative technique to FTBBx during post-lung transplant bronchoscopy. Further studies are needed to determine if larger samples obtained with CPBx translate to an increased diagnostic yield.**

Trial registry: **ClinicalTrials.gov; No.: NA_00052081; URL: clinicaltrials.gov**

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Abbreviations: BOS = bronchiolitis obliterans syndrome CPBx = cryoprobe biopsy; FTBBx = transbronchial biopsy using standard forceps; Spo_2 = saturation of peripheral oxygen

Acute cellular rejection and lymphocytic bronchiolitis have been shown to be major risk factors for the development of bronchiolitis obliterans syndrome (BOS).[1-7] The prevalence of BOS in recipients of lung transplants alive at 5 years is > 50% and is strongly associated with increased mortality, particularly in those patients who develop BOS within 2 years post-transplant.[1] While no cure for BOS is currently available, early accurate detection and treatment of acute

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Affiliations: From the Department of Pulmonary and Critical Care (Drs Yarmus, Akulian, Shah, Merlo, Orens, and Feller-Kopman), Johns Hopkins University, Baltimore, MD; Department of Pulmonary and Critical Care (Dr Gilbert), Pennsylvania State University, Hershey, PA; and Department of Pathology (Dr Illei), Johns Hopkins University, Baltimore, MD.

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Correspondence to: Lonny Yarmus, DO, FCCP, Johns Hopkins University School of Medicine, Section of Interventional Pulmonology, Division of Pulmonary and Critical Care, 1800 Orleans St, Ste 7125M, Baltimore, MD 21205; e-mail: lyarmus@jhmi.edu

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cellular rejection is important in decreasing the risk of progression to BOS.[7]

The current gold standard in evaluating the posttransplanted lung allograft is through flexible bronchoscopy with Radial Jaw forceps (Boston Scientific Corp) for transbronchial lung biopsy (FTBBx).[8] FTBBx is well known to suffer from a lack of quality and quantity due to alveolar, airway, and vascular crush artifact, as well as the limited sample size. Studies aimed at optimizing the diagnostic yield of FTBBx in this patient population recommend that a minimum of 10 biopsy samples should be submitted for pathologic evaluation to ensure adequate sensitivity and specificity.[9] Unfortunately, the risk of bleeding and pneumothorax increases with each attempt at biopsy. These limitations have led to the search for other modalities of diagnosis for acute cellular rejection, lymphocytic bronchiolitis, and infection.

Cryoprobes have been used in the bronchoscopic management of malignant endobronchial disease since the 1970s.[10] The cryoprobe operates using the Joule-Thomson effect, in which compressed gas (most commonly liquid nitrous oxide) undergoes adiabatic expansion and rapidly cools the probe tip to −89°C. This allows for adequate anchoring of the probe to lung parenchyma and biopsy specimen retrieval.

Studies in the use of flexible cryoprobes for cryoprobe endobronchial and cryoprobe transbronchial lung biopsy (CPBx) have shown improvement in diagnostic yield, sample size, and architectural preservation of the biopsy specimen. In addition, these studies have shown complication rates comparable to FTBBx.[11-15] These findings, combined with the need for improved sampling of the posttransplanted lung allograft, appear to make CPBx ideal.

There is a known increased bleeding risk during transbronchial biopsy in recipients of lung transplant and bronchoscopic procedures in patients who have undergone lung transplant are five times more likely to have the procedure aborted secondary to bleeding complications.[16,17] Prior to this study, there were no data investigating the safety of CPBx in the population of patients who have undergone lung transplant. In this study, we performed the first prospective investigation of the use of the cryobiopsy technique in patients who have undergone lung transplant.

Table 1—*Patient Characteristics*

Characteristics	Cryoprobe Study Patients (n = 17)
Total procedures	21
Age, median (range), y	52 ± 13
Sex	
Male patients	12
Indication for lung transplantation	
Sarcoidosis	3
Cystic fibrosis	6
COPD	3
IPF/ILD	4
PHTN	1
Days after lung transplantation, median (range)	387 ± 307

Data presented as No. unless otherwise indicated. ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis; PHTN = pulmonary hypertension.

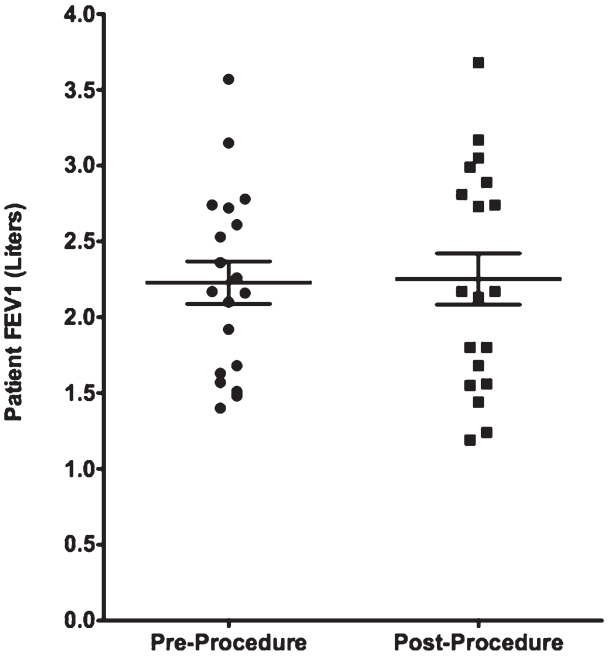

FIGURE 1. Preprocedure and postprocedure FEV_1. Mean ± SEM *P* values are shown.

Materials and Methods

Patients

This protocol was approved by the institutional review board at The Johns Hopkins Hospital, Baltimore, Maryland (IRB NA_00052081). Twenty-one procedures were performed on 17 consecutive patients referred for transbronchial lung biopsy between November 2011 and September 2012. Study inclusion criteria included age > 18 years and bilateral, orthotopic lung transplant undergoing surveillance bronchoscopy. Exclusion criteria included coagulopathy (platelet count < 50,000, international normalized ratio > 1.5), FEV_1 < 0.8 L, diffuse bullous disease, hemodynamic instability, and severe hypoxemia (Pao_2 < 55 mm Hg or saturation of peripheral oxygen (Spo_2) < 92% on room air). No patients met exclusion criteria during the pilot time period. All patients underwent preprocedure and postprocedure pulmonary function testing (spirometry, lung volumes, diffusing capacity) and had preprocedure coagulation studies checked.

Procedure

Given the potential risk of bleeding in this patient population, the first 10 procedures were conducted in the operating room via rigid bronchoscopy. Patient monitoring included oxygen saturation, heart rate, BP, and the cardiac-rhythm strip. IV sedation with propofol and paralytics was administered by an attending

Table 2—*Procedural Events*

Biopsy Type	Forceps	Cryoprobe
Total procedures	21	21
Bleeding grade		
0	10	9
1	11	11
2	0	1
3	0	0
4	0	0
Perioperative pneumothorax	0	0
Postoperative pneumothorax	0	0
Delayed pneumothorax	1	1
Bronchial blocker inflation	0	0
Respiratory distress	0	0
Postoperative admission	0	0

Data presented as No.

anesthesiologist. All patients were intubated using a 13.2-mm, rigid bronchoscope (Bryan Corp). Jet ventilation was used for each procedure.

After a thorough airway inspection with a flexible bronchoscope (Olympus XT160; Olympus America Inc), the mainstem bronchus of the lung to be sampled was intubated with the rigid bronchoscope. A deflated bronchial blocker (Arndt Endobronchial Blocker; Cook Corp) was placed preemptively as part of our predefined, pilot safety-study design. A standard C-arm fluoroscope (Koninklijke Philips Electronics NV) was positioned and with the flexible bronchoscope in wedge position in the segmental bronchus, 10 FTBBx (Radial Jaw 3 Pulmonary Biopsy Forceps; Boston Scientific Corp) samples were obtained in the standard fashion. The presence of a pneumothorax was then assessed with fluoroscopy. Following this, five CPBx specimens were obtained under fluoroscopic control by passing the 1.8-mm cryoprobe connected to the cryotherapy system (ERBE Elektromedizin GmBH) through the working channel of the flexible bronchoscope. The activating pedal on the cryoprobe unit was depressed for 3 s, after which the bronchoscope, cryoprobe, and biopsy specimen were removed en bloc through the rigid bronchoscope. Again, fluoroscopy was performed at the conclusion of collecting the final CPBx sample to assess for pneumothorax. Both methods of transbronchial biopsy were directed into the same regions under bronchoscopic and fluoroscopic guidance. All samples were fixed in formaldehyde and immediately transported to pathology.

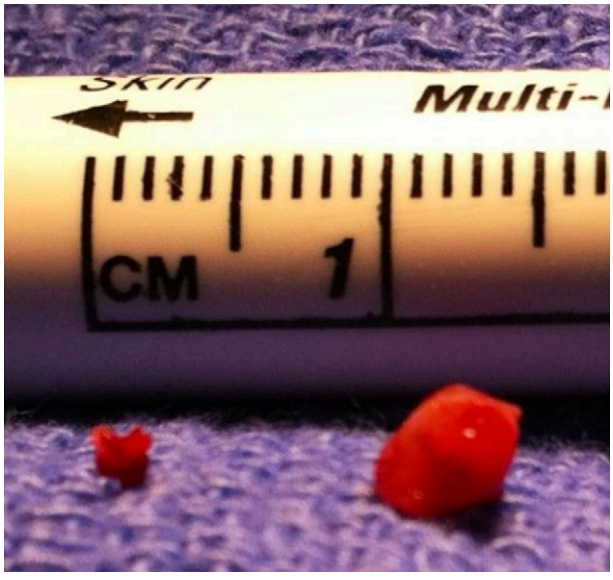

FIGURE 2. Single, transbronchial biopsy specimen collected using standard forceps is smaller than a gross pathology specimen collected using a cryoprobe.

The last 11 procedures on patients in the pilot safety study were performed in the endoscopy suite. IV deep sedation with propofol was administered by an attending anesthesiologist, with monitoring identical to that of the first 10 procedures. Bronchoscopy was performed via a size 4 or 5 laryngeal mask airway (LMA North America Inc) and patients breathed spontaneously throughout the procedure. A bronchial blocker was not placed in these 11 patients but was readily available in the endoscopy suite per our current standard of care.

During and after the procedure, patients were monitored for complications including bleeding, hemodynamic instability (heart rate <60 or >120, systolic BP >150 or <90, diastolic BP >100 or <50), respiratory compromise ($Spo_2 < 92\%$ on room air), and pneumothorax with intraprocedure fluoroscopy as well as a chest radiograph performed 1-2 h postprocedure. Bleeding was categorized on an adapted grading system[18]: grade 0, traces of blood not requiring suctioning; grade 1, bleeding requiring suction to clear; grade 2, bleeding requiring wedging of the biopsied segment with the flexible bronchoscope and/or iced saline; grade 3, bleeding requiring further intervention such as inflation of the bronchial blocker and/or the use of fibrin sealant; grade 4, bleeding requiring surgical intervention, cardiopulmonary instability, transfusion of packed RBCs, cessation of the procedure, and/or admission to the ICU.

Specimen Processing and Evaluation

All biopsy specimens were placed in formalin and later treated with hematoxylin-eosin and Movat stains and prepared in paraffin blocks for evaluation by a dedicated lung pathologist for total sample area (mm^2), alveolated area (mm^2), percent open alveoli, percent of artifact-free lung parenchyma, presence of crush or freezing artifact, and presence of rejection that would be graded A and/or B.

Statistical Analysis

All data were analyzed using GraphPad (GraphPad Software Inc) or Stata (StataCorp LP) statistical packages. χ^2 testing was used when comparing proportions. Mann-Whitney and Wilcoxon signed-rank test were used when appropriate to compare mean values. P value $<.05$ was considered significant.

RESULTS

Twenty-one procedures were performed in 17 patients (12 male and 5 female patients) with a median (range) age of 52 ($\pm$ 13) years. The median time since lung transplant was 387 ($\pm$ 307) days. The indication for lung transplant included cystic fibrosis, COPD, interstitial lung disease/idiopathic pulmonary fibrosis, sarcoidosis, and pulmonary hypertension (Table 1). No significant difference in mean FEV_1 preoperatively and postprocedure was noted (Fig 1).

Ten FTBBx and five CPBx specimens were successfully obtained from all 21 cases. There were no clinically or statistically significant differences in bleeding

between the two biopsy techniques (Table 2). There were no grade 3 or 4 bleeding incidences in either the CPBx or FTBBx group. No cases required bronchial-blocker inflation in either group. There were no perioperative pneumothoraces on fluoroscopy and no immediate postoperative pneumothoraces in either group. One patient developed chest pain 3 days after the procedure and a chest radiograph confirmed a delayed pneumothorax. She was evaluated at an outside hospital and although she had no hemodynamic or respiratory symptoms, a chest drain was placed electively, resulting in complete lung reexpansion. Detailed review of the pathologic specimens revealed no evidence of pleural tissue in either the CPBx or FTBBx samples. There were no cases of respiratory distress or respiratory failure in either group. No patients required any mechanical ventilatory support postprocedure. There were no postprocedure admissions or prolonged observations in either group.

Mean specimen size was significantly larger with the CPBx technique than the FTBBx procedure (Figs 2, 3). There was a significant difference between the two techniques in terms of the percentage of sampled tissue containing open alveoli (Fig 4). There was no clinical evidence of crush artifact on any CPBx samples, whereas all FTBBx samples had significant amounts of crush artifact evident on pathologic review (Fig 5). Despite potential concerns regarding potential freezing artifact from the cryoprobe, there was no pathologic evidence of freezing artifact seen on any CPBx sample.

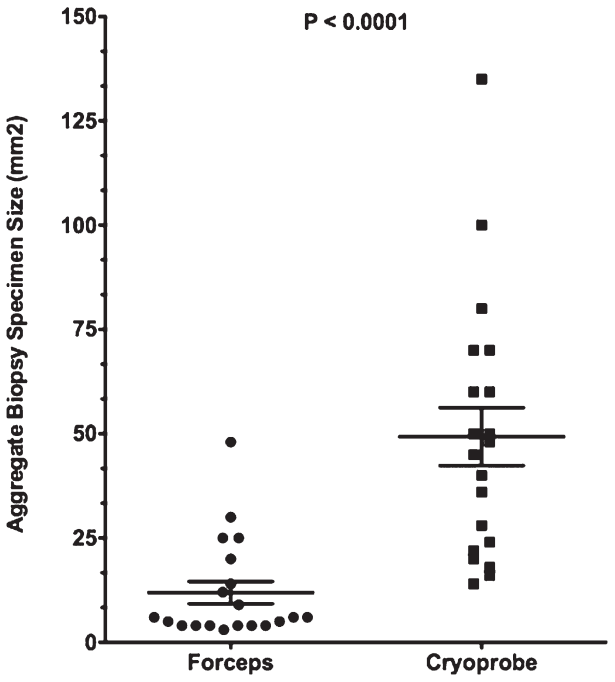

FIGURE 3. Aggregate biopsy specimen size (mm^2). Mean ± SEM *P* values are shown.

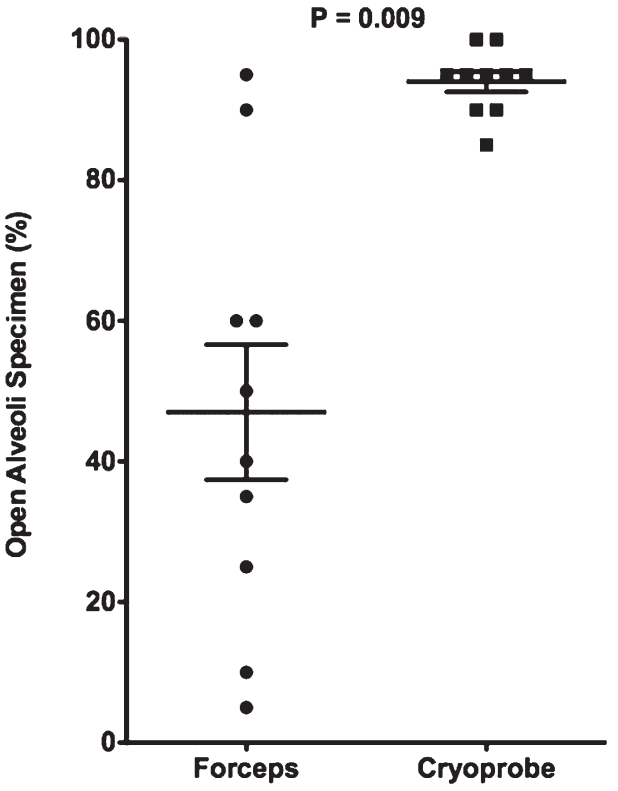

FIGURE 4. Measure of crush artifact with percent open alveoli on pathologic review. Mean ± SEM *P* values are shown.

DISCUSSION

Our study provides the first data demonstrating that the use of CPBx in patients after lung transplantation is safe and provides significantly larger specimens for pathologic evaluation. The goals of transbronchial biopsy in recipients of lung transplants are to safely and effectively biopsy the allograft to assess for pathology not otherwise evaluable via clinical history, radiology, and pulmonary function testing, with the aim of identifying early pathology and preventing the development of BOS.[9,19-21] There is a broad variance in the practice of FTBBx among lung transplant centers,[22] as well as a sense that due to insufficient quality and quantity of biopsy samples, FTBBx leads to underdiagnosing of acute cellular rejection.[11,23]

The present study is the first prospective investigation of the use of CPBx in the population of patients who have undergone lung transplant. Despite our concerns regarding an increased bleeding risk in this group and a higher risk of bleeding with larger sample-size acquisition, there was no attributable risk identified in this pilot study. Our initial methodology using a rigid bronchoscopic approach in the operating room allowed optimal patient safety. Given the lack of any significant bleeding in the first 10 patients, we were able to transition to an endoscopy suite-based procedure to further assess the safety of this procedure

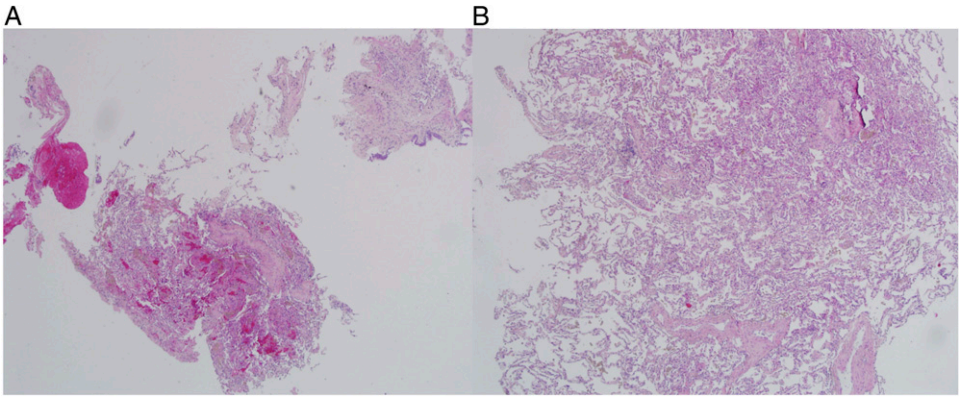

FIGURE 5. Histologic transbronchial biopsy sample. A, Using standard forceps. B, Using cryoprobe. Note the crush artifact and blood on the forceps biopsy sample vs the cryoprobe biopsy sample.

under more-available, flexible bronchoscopic means. Using these standard bronchoscopic techniques, we were again able to show an excellent safety profile.

To our knowledge, this is the first pilot safety study investigating the use of the cryoprobe vs transbronchial biopsy forceps for parenchymal lung biopsies. There is one published study that has investigated the use of the cryoprobe for obtaining parenchymal samples in patients with diffuse lung disease; however, none of the patients had received a lung transplant. That study was performed retrospectively and was further limited by minimal biopsy material in that only one FTBBx and one CPBx were performed for each procedure. The study also did not track bleeding or complication events.[11] One other study examined the feasibility of obtaining cryoprobe samples in 10 patients, but no comparison was made between modalities and no significant safety data were reported.[23]

Our study does have some limitations. Although the study was prospective in design, given the gross difference visualized in specimen size, it was not possible to blind the pathologist's review. The initial unknown risks and the tenuous clinical status of patients early after lung transplantation influenced the decision to exclude patients < 3 months after lung transplant and may mask potential risk in this subset of patients. Although there were no perioperative or postoperative complications, we were unable to attribute either biopsy sampling technique as the cause of the delayed pneumothorax that occurred in one patient at 72 h. Despite detailed pathologic review of the samples, no pleural tissue was observed in either the CPBx or FTBBx specimens. The trial was designed as a pilot trial with safety and feasibility as the primary end point.

Our results show that CPBx is a safe and feasible technique for use in patients who have undergone lung transplant. We report no clinically significant complications attributed to either biopsy technique. There were no significant postoperative complications or effects on lung function in either group. We were also able to show a statistically significant improvement in biopsy size and architectural preservation, as well as a decrease in the amount of crush artifact on pathologic review.

The importance of this research cannot be understated in that acute cellular rejection and lymphocytic bronchiolitis after lung transplant have been repeatedly shown to be major risk factors in the development of chronic rejection, or BOS, and increased mortality. Additional studies are needed to confirm our preliminary results indicating that transbronchial CPBx will produce larger samples without crush artifact, allowing a more accurate diagnosis of acute cellular rejection, leading to earlier therapy and, hopefully, lower rates of BOS in the long term.

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Author contributions: Dr Yarmus had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Dr Yarmus: contributed to the study design, performing the procedures in the study, statistical review, writing the manuscript and served as principal author.
Dr Akulian: contributed to the study design, performing the procedures in the study, statistical review, and writing the manuscript.
Dr Gilbert: contributed to the study design, performing the procedures in the study, statistical review, and writing the manuscript.
Dr Illei: contributed to the study design, statistical review, and writing the manuscript.
Dr Shah: contributed to the study design, statistical review, and writing the manuscript.
Dr Merlo: contributed to the study design, statistical review, and writing the manuscript.
Dr Orens: contributed to the study design, statistical review, and writing the manuscript.
Dr Feller-Kopman: contributed to the study design, performing the procedures in the study, statistical review, and writing the manuscript.
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