

Transbronchial Cryobiopsy: A New Tool for Lung Biopsies

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Key Words

Transbronchial biopsy · Cryobiopsy · Interventional bronchoscopy · Interstitial lung disease

Abstract

Background: Specimens from transbronchial lung biopsies lack sufficient quality due to crush artifact and are generally too small for diagnosis of diffuse lung diseases. Flexible cryoprobes have been shown to be useful in therapeutic bronchoscopy. We introduce a novel technique for obtaining lung biopsies bronchoscopically, using a flexible cryoprobe. **Objectives:** The purpose of this study was to show the feasibility of using a cryoprobe to obtain lung biopsies during flexible bronchoscopy. **Methods:** Forty-one patients with radiographic signs of diffuse lung disease were selected for transbronchial biopsy. During flexible bronchoscopy, conventional transbronchial biopsies using forceps were done first. Then a flexible cryoprobe was introduced into the selected bronchus under fluoroscopic guidance. Once brought into position, the probe was cooled and then retracted with the frozen lung tissue being attached on the probe's tip. The tissue was processed for histology. After establishing a diagnosis, the specimen area was measured using a digital morphometry system. **Results:** We evaluated the biopsy sam-

ples of 41 patients. The mean specimen area was 5.82 mm² (0.58–20.88 mm²) taken by forceps compared to 15.11 mm² obtained using the cryoprobe (2.15–54.15 mm², $p < 0.01$). Two patients had a pneumothorax which resolved with tube thoracostomy. Biopsy-associated bleeding did not require any intervention. Transbronchial cryobiopsy contributed in a substantial number of cases to a definitive diagnosis. **Conclusions:** Transbronchial cryobiopsy is a novel technique which allows to obtain large biopsy samples of lung parenchyma that exceed the size and quality of forceps biopsy samples. Prospective trials are needed to compare this technique with surgical lung biopsy for diagnosis of diffuse lung diseases.

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Introduction

Transbronchial biopsies allow harvesting tissue from the periphery of the lung for diagnosis of diffuse lung diseases. This is usually done under fluoroscopic guidance, targeting the segment of interest. Transbronchial

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Fig. 1. A flexible cryoprobe is shown. The metal tip, which can be visualized under fluoroscopic control, is enlarged.

biopsies play a minor role in the diagnosis of idiopathic interstitial pneumonias [1]. They are largely used to rule out granulomatous diseases or infections. Although the contribution of transbronchial biopsy for diagnosis of idiopathic pulmonary fibrosis (IPF) was shown in 1 study to be close to 32%, surgical lung biopsy is considered the gold standard [2]. This kind of biopsy is done mostly as video-assisted thoracoscopic surgery, which carries a substantial risk of morbidity and mortality [3].

Surgical lung biopsies being the gold standard for diagnosis of idiopathic interstitial pneumonias is largely a function of the size of the surgical specimens. Transbronchial forceps biopsies, in addition to being small, are also subject to crush artifacts. This report shows a novel way of obtaining specimen of lung parenchyma with flexible bronchoscopy using a flexible cryoprobe.

Cryosurgical techniques have been used in the airways since 1968 [4].

The majority of cryoapplication has been in the palliative treatment of obstructing endobronchial tumors. However, treatment of early superficial bronchogenic carcinoma has been attempted with cryotherapy [5]. Endobronchial cryotherapy with a flexible bronchoscope was first described in the United States in 1996 by Mathur et al. [6], who reported relief of obstruction in 90% of their patients (n = 20). Since then, the experience with endobronchial cryosurgery has broadened, with several studies reporting its favorable safety, effectiveness and cost profile [7]. Flexible cryoprobes have found their position in pulmonary medicine when managing tumorous and nonmalignant endobronchial obstruction [8, 9]. The cryosurgical equipment consists of the console, cryogen

and the cryoprobe (fig. 1). The cryosurgical equipment operates by the Joule-Thompson effect, which dictates that a compressed gas released at high flow rapidly expands and creates a very low temperature. The advantage of the cryoprobe is that large pieces of tissue can be extracted with the freeze-thaw cycle. In the early days, during cryotherapy the cryoprobe was attached on the endobronchial lesion and frozen in order to induce necrosis. The probe was removed, leaving tissue behind. After several days, a clean-up procedure was needed to remove the necrotic tissue. The use of flexible cryoprobes was modified towards cryorecanalization. With this technique, the cryoprobe is removed out of the bronchial system while still being frozen, with the tissue attached on the frozen probe's tip. Efficiency and the advantage of an immediate effect were shown previously [8]. Experience with transbronchial cryobiopsy is limited [10, 11]. This study shows a new promising way to use cryoprobes in order to obtain specimens from the lung parenchyma during flexible bronchoscopy. Points of interest were feasibility and sample size compared to forceps biopsies and safety.

Patients and Methods

Patients

A retrospective data evaluation was done of consecutive patients that underwent transbronchial forceps biopsies and cryobiopsies during the same procedure from July 2006 to December 2006. A total of 41 patients with diffuse lung diseases as seen on CT of the thorax were eligible for review. According to the standard of care protocol in the performing institutions, patients had to be at least 18 years old, were required to have a pO₂ of at least 60 mm Hg under oxygen delivery of 2 liters/min or lower flow, a normal blood count as well as normal coagulation parameters. Patients who had pulmonary arterial hypertension with right ventricular systolic pressure of more than 40 mm Hg by echocardiography were excluded. Written informed consent for bronchoscopy and tissue sampling with a cryoprobe was obtained from all patients. Analysis of the data was approved by an institutional review board.

The Cryoprobe

A flexible cryoprobe measuring 90 cm in length and 2.4 mm in diameter was used (ERBE, Germany, fig. 1). The probe was cooled with nitrous oxide which allowed to decrease the temperature in the probe's tip to -89°C within several seconds.

The Procedure

All procedures were carried out during flexible bronchoscopy. The patients were deeply sedated with intravenous propofol and intubated with a spiral armored endotracheal tube (Bronchoflex®, Teleflex). Oxygen was insufflated continuously through this tube; spontaneous breathing was maintained during the whole procedure. Oxygen saturation, blood pressure, ECG and

Table 1. Number of cases diagnosed using different biopsy techniques

Definitive diagnosis	History, lung function, CT, forceps biopsy, cryobiopsy	History, lung function, CT, forceps biopsy	History, lung function, CT ^a
IPF/UIP	15	11	11
NSIP	10	3	0
Desquamative interstitial pneumonia	3	3	0
Pulmonary lymphangioleiomyomatosis	1	1	0
Hypersensitivity pneumonitis	3	2	1 ^b
Sarcoidosis	6	4	2 ^b
Pharmacologically induced pneumonitis	1	0	0

^a Two cases (one IPF and one NSIP) were diagnosed with surgical lung biopsy.

^b Including laboratory testing and bronchoalveolar lavage.

transcutaneous carbon dioxide partial pressure were monitored continuously.

The bronchopulmonary segment for biopsy was determined prior to the procedure based on the CT of the chest. During flexible bronchoscopy, the bronchoscope was introduced into the selected bronchus. Under fluoroscopic guidance, the biopsy instrument was navigated into the target area. A transbronchial forceps biopsy was done first. Particular caution was given to the position of the biopsy area: it required that the biopsy instrument be placed perpendicular to the chest wall to assure an accurate evaluation of the distance to the thoracic wall by fluoroscopy. Then a cryobiopsy was obtained in the same segment. The cryoprobe was introduced into the selected area under fluoroscopic guidance. A distance of approximately 10 mm from the thoracic wall was considered optimal. Once brought into position, the probe was cooled for approximately 4 s, then it was retracted with the frozen lung tissue being attached on the probe's tip. The frozen specimen was thawed in saline and fixed in formalin. Each patient had at least 1 biopsy using each technique. The number of biopsies taken was dependent upon the investigator's decision.

Within 2 h after the procedure, a chest X-ray was done to exclude pneumothorax. The length of the procedure was the time from intubation to extubation of the patient. The intensity of bleeding was distinguished as controllable by suction with the flexible bronchoscope and the necessity of tamponade or other interventions.

Processing the Histological Samples

Preparation of Specimens

Specimens were fixed in 4% formalin and embedded in paraffin. Slices measuring 3 μ m were made and mounted onto slides. Hematoxylin-eosin, elastica van Gieson and PAS staining were done.

Histological Evaluation

Each slide was evaluated by a pathologist. Clinical features and the radiological pattern of the suggested lung disease were submitted to the pathologist. Definitive diagnosis was based on clinical, radiographic and pathologic findings including the clinical course over a period of at least 6 months [1].

Morphometry

Each slide was visualized and analyzed using a digital morphometry system. The area of the histologic sample was calculated. If there was more than 1 specimen, the largest one was taken for measurement. Sample size was measured in mm²; the results were compared using the rank sum test.

Results

Procedure

Biopsies obtained from 41 patients were evaluated. All patients underwent the procedure. On average, 2 biopsies were taken by cryoprobe and by forceps. The average duration of the procedure was 26 min (19–42 min). None of the patients required prolonged mechanical ventilator support after the procedure.

None of the patients needed interventional bleeding control, such as tamponade, double lumen intubation or surgery. Two patients (4.87%) sustained pneumothorax after the procedure, requiring drainage using a chest tube over 3 days. Both patients were discharged after 3 days with complete reexpansion.

Histology

Seventy-four cryobiopsy samples and 82 forceps biopsies were collected and suitable for analysis (fig. 2). Preparation and staining were possible in all of the samples regardless of the technique of tissue acquisition. All samples acquired by the cryoprobe contained lung tissue (fig. 3). Crush artifacts were not seen, therefore the alveoli were not compressed and the tissue architecture was patent. Cellular structures were also preserved, as far as could be seen in light microscopy.

Fig. 2. Macroscopic photographs of biopsy samples taken by forceps (a) and by cryoprobe (b).

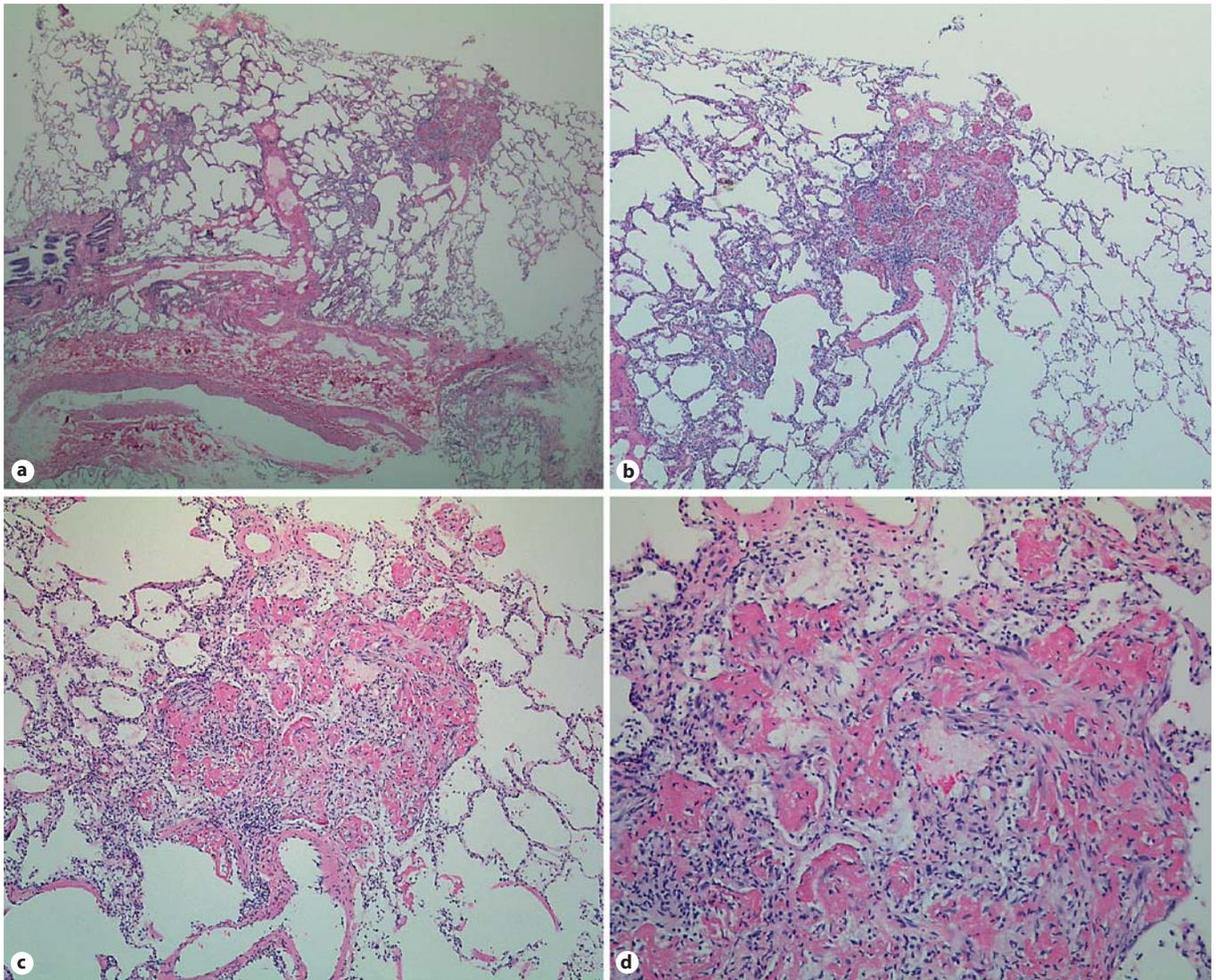
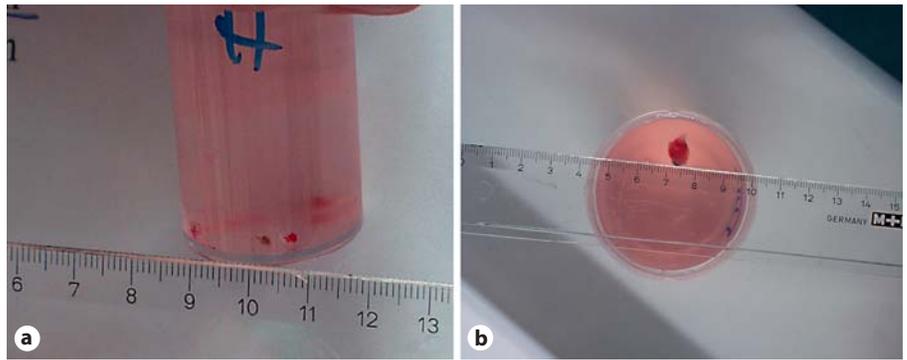


Fig. 3. a Histologic sample of a transbronchial biopsy using the cryoprobe. The patient was diagnosed to suffer from a medication-induced pneumonitis. The sample fills out the histologic slide and shows a large portion of lung tissue as well as bronchial

and vascular structures. **b–d** Incremental increase of magnification shows the preserved condition of alveolar structures. Inflammatory manifestations are visible within the lung tissue.

In 39 of 41 patients, a definitive diagnosis could be made upon adding information from history, noninvasive testing and biopsy samples. Two patients underwent surgical lung biopsy for definitive diagnosis. The distribution of the definitive diagnosis is shown in table 1. In 30 patients, the definitive diagnosis of idiopathic interstitial pneumonia was made; 2 of them required surgical lung biopsy. In patients having a definitive diagnosis of IPF/usual interstitial pneumonia ($n = 16$), forceps biopsy did not influence the clinical diagnosis of IPF, whereas cryobiopsy made a definitive diagnosis possible in a total of 15 patients; 1 patient required surgical lung biopsy. In patients with nonspecific interstitial pneumonia (NSIP; $n = 11$), forceps biopsies added in 3 cases further information to the radiological pattern and the clinical course, the cryoprobe led to the definitive diagnosis in a total of 10 cases and 1 patient had surgical lung biopsy. The contribution of each sampling technique is shown in table 1.

One sample was taken for analysis with each biopsy technique, 41 in total with each technique. All 82 samples could be evaluated morphometrically. Analysis of the samples showed a median sample area on the histologic slide of 5.82 mm^2 ($0.58\text{--}20.88 \text{ mm}^2$) taken by forceps compared to 15.11 mm^2 obtained using a cryoprobe ($2.15\text{--}54.15 \text{ mm}^2$, $p < 0.01$).

Discussion

This study shows a new way of obtaining transbronchial lung biopsies using flexible cryoprobes. All patients who were included and evaluated retrospectively had transbronchial biopsies using a cryoprobe and forceps. Applying this new technique was shown to be feasible; representative lung tissue could be obtained in all patients. Focusing mainly on the biopsy sample size, a clear advantage was shown for those taken by the cryoprobe. Currently, there is no data in the literature concerning the specimen size of transbronchial biopsies, since earlier studies focused only on the diagnostic yield of the technique itself [12, 13].

The diagnostic yield of each biopsy technique was not of primary interest, and the heterogeneous cohort of different lung diseases additionally compromised this endpoint. Nevertheless, some cautious statements can be drawn from the data: in a significant number of cases, transbronchial cryobiopsy contributed substantially to the definitive diagnosis. Especially in idiopathic interstitial pneumonia, transbronchial cryobiopsy added important information. The size of the samples obtained seems

to increase the diagnostic yield immensely compared to forceps biopsies. In the presented group of patients, forceps biopsies – especially in diagnosing IPF and NSIP – showed a remarkably low diagnostic yield compared to existing data [2]. One explanation might be the low number of samples obtained, which additionally underlines the significance of the sample size taken by cryoprobe. It is interesting to point out 1 case of a sirolimus-induced pneumonitis which was diagnosed from a cryobiopsy sample. Its histological pattern is shown in figure 3. The authors contend that surgical lung biopsy being a gold standard for the diagnosis of interstitial lung disease is largely a function of the size of the tissue sampled. While it is clear that conventional transbronchial forceps biopsies are too small to describe the histologic characteristics of various interstitial lung diseases, it is not clear whether we need the size of tissue samples obtained by surgical lung biopsies. Transbronchial cryobiopsies are much larger than conventional forceps biopsies but smaller than surgical lung biopsies. In this study, we set out to determine whether this tissue size is adequate for reliably diagnosing diffuse lung diseases.

Safety clearly is one of the most important concerns when introducing a new technique. Significant bleeding and the occurrence of pneumothorax represent the most important potential complications. Although having a potentially large defect in the lung parenchyma, the occurrence of pneumothorax and bleeding was low compared to data from forceps biopsies [14]. Bleeding never necessitated any bronchoscopic interventions except suction using the flexible bronchoscope. The hemostatic effects of cooling probably contributed to the low incidence of significant bleeding.

The mechanism of action when obtaining biopsy samples is not clear. As shown previously, the tissue surrounding the probe's tip is being frozen and thereby can draw the surrounding tissue [8]. Acquiring biopsies using flexible cryoprobes has been shown to be effective in the central airways [10].

The authors suggest that, for safety reasons, all patients undergoing this procedure should be intubated to ensure airway access. Biopsies should always be performed under fluoroscopic guidance. They should be taken only when there is sufficient distance from the probe tip to the chest wall which is assessable by fluoroscopy. Therefore the area of biopsy has to be perpendicular to the chest wall during fluoroscopy.

The authors do not claim superiority of transbronchial cryobiopsy over the diagnostic yield of other techniques, especially surgical lung biopsy, but show a case

series which is thought of as a feasibility study. The overall diagnostic yield is high but it is important to point out that all clinical data such as the clinical course and radiological patterns were taken into consideration for definitive diagnosis. Additionally, lung diseases such as sarcoidosis and hypersensitivity pneumonitis were included, which can often be diagnosed in samples taken from forceps biopsies.

At this time, transbronchial biopsies play only a subsidiary role in diagnosing diffuse lung diseases, mostly

due to the small size of the biopsy samples. Another major concern is the reduced quality of the samples due to atelectasis of the specimens [2].

Large transbronchial biopsy samples of lung tissue of good quality might immensely contribute to the diagnostic algorithm of parenchymal lung disease. Prospective comparison trials with surgical lung biopsies are needed to establish transbronchial cryoprobes as a diagnostic modality for diffuse lung diseases.

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