



Original Article

Transbronchial Lung Biopsy Using Cryoprobes

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ABSTRACT

Background and objectives: Transbronchial lung biopsy (TBLB) is a bronchoscopy procedure used to obtain peripheral lung tissue. Small size samples and artefacts lead to variable, and usually poor, diagnostic yield. The use of cryoprobes may enable larger size and better quality biopsy samples to be obtained. The purpose of this study was to evaluate the feasibility of TBLB with cryoprobes and analyse the histological quality of samples obtained.

Patients and methods: We selected 10 patients with interstitial lung disease who were suitable for TBLB. A cryoprobe (Erbokryo CA®, Erbe, Germany) was introduced through the bronchoscope work channel. Then, under fluoroscopic control, the cryoprobe was placed in an area of the peripheral lung previously selected according to CT findings. A temperature of -89.5°C was applied for 3s and the cryoprobe and bronchoscope were removed with the frozen lung sample attached to the probe. The procedure was performed under sedation and the patient was intubated to allow bronchoscope and cryoprobe removal. Safety, duration of the procedure and histological findings has been evaluated.

Results: There were 10 patients (64 ± 8 years, 6 males). Procedure length was 35min. The specimen area was 9.5mm^2 (range 3 to 25mm^2) and the mean number of alveolar spaces was 29.62. No pneumothorax was registered. 6/10 patients had mild post-biopsy bleeding controlled with standard bronchoscopy measures.

Conclusions: The use of cryoprobes for TBLB may become an alternative technique to increase diagnostic yield.

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Utilización de criosondas para la realización de la biopsia pulmonar transbronquial

RESUMEN

Introducción: La biopsia pulmonar transbronquial (BPTB) es una técnica broncoscópica indicada en el estudio de las enfermedades pulmonares difusas, cuyo rendimiento diagnóstico es variable debido, en parte, al pequeño tamaño de las muestras obtenidas. La utilización de criosondas en la práctica de la BPTB podría permitir obtener muestras tisulares de mayor tamaño y mejor calidad. El presente trabajo tiene como objetivos describir la metodología de la técnica y su implantación en nuestro centro, así como analizar los resultados de seguridad e histológicos en los primeros pacientes.

Pacientes y métodos: Se incluyó a 10 pacientes tributarios de BPTB para estudio de neumopatía difusa. De un modo equiparable al método convencional, la criosonda (Erbokryo CA®, Erbe, Alemania) se introduce a tra-

Palabras clave:

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vés del videobroncoscopio y se dirige hacia regiones pulmonares periféricas, donde la aplicación de frío permite obtener una muestra de tejido pulmonar congelado que queda adherido al extremo de la criosonda. Se registraron la duración del procedimiento y las complicaciones durante éste, y se evaluó la calidad de las muestras obtenidas.

Resultados: La duración media (\pm desviación estándar) del procedimiento fue de 35 ± 11 min. Las muestras presentaban un área media de $9,5 \text{ mm}^2$ (rango: $3\text{--}25 \text{ mm}^2$), con un número medio de espacios alveolares conservados de 29,6. Ningún paciente presentó neumotórax. En 6 de los 10 pacientes se registró hemorragia tras la biopsia, que en ningún caso obligó a interrumpir el procedimiento.

Conclusiones: La utilización de criosondas es viable para la realización de la BPTB y en el futuro podría mejorar el rendimiento de la técnica convencional.

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Introduction

Diffuse pulmonary diseases comprise a heterogeneous group of complaints with different prognostic and therapeutic implications, which make a specific histological diagnosis necessary in most cases.¹ To study these diseases, transbronchial lung biopsy (TBLB) using flexible bronchoscopy, first described by Levin et al² in 1974, is the technique of choice for obtaining lung parenchyma.^{3,4} However, a definitive diagnosis is often not possible after a histological analysis of the samples obtained using TBLB, so more aggressive and expensive surgery is necessary. Amongst other factors, the variability in the diagnostic yield of TBLB is due to the small size of the biopsies obtained and the alterations caused by the pressure of the forceps on the biopsy tissue, both of which limit the correct anatomopathological analysis of samples.

On the other hand, cryotherapy has been used in bronchoscopy for many years, and its main use is the treatment and excision of endobronchial lesions, particularly in cases of bronchial obstruction.⁵ In studies assessing the histological material obtained using cryoprobes in cases of endobronchial tumours, the samples were found to be larger than those obtained with conventional forceps.^{6,7} This has led to considering the possibility of using cryoprobes for performing TBLB as a new, improved alternative to the classic method used to study diffuse lung diseases. It could increase diagnostic yield without increasing risks, and avoid the added cost of surgery. However, there is only a very limited amount of information about this technique and, to our knowledge, no centre in our country uses it.

This paper describes the method used to perform TBLB with a cryoprobe and its implementation in our hospital, and analyzes the first histological and safety results obtained following its use on patients with diffuse lung diseases.

Patients and Methods

A prospective study was carried out in 10 patients with indication for TBLB to study a diffuse lung disease. The patients selected were assessed in the pneumology outpatients department. All the patients had a haemogram with a coagulation study, an echocardiogram, a high resolution CT scan of the chest; a study of lung function, including spirometry, was carried out, and lung volumes and carbon dioxide diffusion were established. The study protocol was approved by the Ethical Committee for Clinical Research of the Hospital de Sant Pau (Barcelona) prior to beginning and all the patients gave their informed consent.

The surgical procedures were carried out in a conventional operating theatre. Patient monitoring and sedation were performed by an anaesthesiologist. Oxyhaemoglobin saturation, blood pressure, heart and breathing rates were recorded, and patients were monitored with an electrocardiogram and capnography. Local anaesthesia of the upper airway was performed with topical lidocaine instillation. The drugs used for sedation were remifentanyl ($0,05\text{--}0,1 \mu\text{g}/\text{kg}^{-1}/\text{h}^{-1}$) and propofol ($3\text{--}6 \text{ mg}/\text{kg}^{-1}/\text{h}^{-1}$) in perfusion. The patients were

intubated under bronchoscopic control with a flexible banded tube (Bronchoflex 7.5mm, Rüschi®) which allowed spontaneous breathing to be maintained and high frequency ventilation to be carried out if necessary. Endoscopic exploration of the bronchial tree was performed through the endotracheal tube using a video bronchoscope (BF 260-T, Olympus®) and a bronchoalveolar lavage was then performed in the selected area, in accordance with the information from the chest CT scan. Subsequently, directed under fluoroscopic control to the previously selected area of the lung, the transbronchial biopsy was then performed using a cryoprobe (Figure 1).

The cryoprobe used was a flexible probe, 2.4mm in diameter and 900mm in length, which was connected to the cryotherapy equipment (Erbokryo® CA, Erbe, Germany).

The tissue is frozen following the Joule-Thomson principle, using gas decompression (nitrous oxide) at the tip of the probe. Contact of the probe with the tissue involves high power freezing (-89°C) which, due to the characteristics of the probe, has stability to traction.

Similar to the technique used to perform transbronchial biopsies, the cryoprobe was introduced through the flexible bronchoscope work channel. Cold was applied for 3 s and then the cryoprobe with the frozen sample attached to the tip was removed, along with the videobronchoscope. Samples were sent to the Anatomical Pathology Department in a formaldehyde solution. We tried to obtain a minimum of 2 biopsies from each patient, although this number varied depending on the patient's tolerance or the onset of complications, or at the bronchoscopist's discretion.

The duration of the procedure was recorded, along with the complications observed, which included: bleeding, pneumothorax

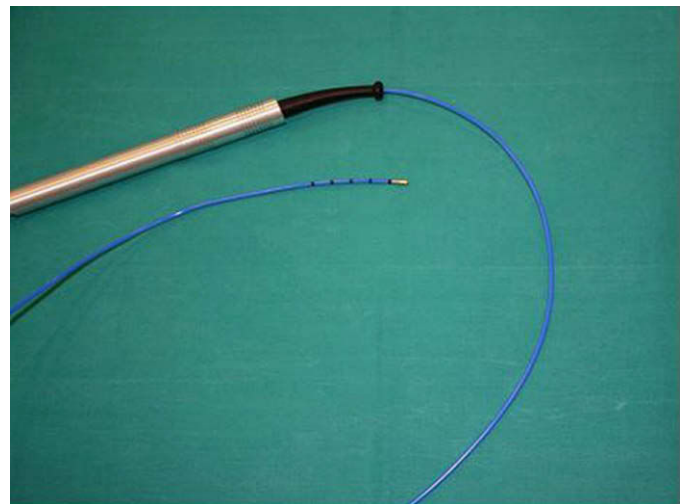


Figure 1. Instrument used to perform cryobiopsies: flexible cryoprobe (Erbokryo® CA, Erbe, Alemania), 2.4mm in diameter and 900mm in length.

and acute respiratory failure (arterial oxygen pressure < 60mmHg and carbonic anhydride arterial pressure > 45mmHg after the operation). Depending on its severity, the pulmonary bleeding was classified as: grade 1, mild bleeding not requiring endoscopic intervention; grade 2, moderate bleeding which stops in under 3 min after endoscopic intervention (bronchial occlusion-collapse and/or instillation of cold saline); and grade 3, severe bleeding which cannot be controlled with bronchoscopic measures, causing haemodynamic or respiratory instability and making it necessary to interrupt the procedure.

As well as the usual description of histological changes, the anatomopathological study assessed the sample for the following characteristics: size (diameter and area), number of alveolar spaces, the percentage of viable lung parenchyma cells for histological study, defined as the representative percentage of alveolar structures in the sample, and the percentage of well-preserved, artefact-free lung parenchyma. A points scale in accordance with quartiles used in previous studies⁶ was used for the histological evaluation of the samples. According to the diagnostic approximation established from the findings observed in the anatomopathological study, the samples were classified as a positive diagnosis, probable diagnosis, a non-diagnosed sample and insufficient material. After the procedure, the patient was extubated and kept under observation for 2-3 h, after which time he was discharged, if there were no complications. Twenty-four hours after the procedure the patient was contacted by telephone to collect information regarding possible symptoms or complications.

The study was performed following the ethical guidelines for clinical research and assays in the Declaration of Helsinki, and it was approved by the Ethics Committee for Clinical Research at the Hospital de la Santa Creu i Sant Pau (Barcelona).

For the statistical analysis of the data, we used the SPSS program for Windows®, version 14.

Table 1
Clinical and functional variables of the 10 patients studied

Age (years)	64 ± 8.4
Sex (male/female)	6/4
FVC (% pred.)	73 ± 21.1
FEV (% pred.)	80 ± 20.1
FEV ₁ /FVC	81 ± 7.4
TLC (% pred.)	78 ± 12.3
DLCO (% pred.)	56 ± 12.1
DLCO/AV (% pred.)	79 ± 17.3

The data are presented as mean ± standard deviation, or number of patients when it is sex. número de pacientes en el caso del sexo. DLCO/VA: diffusing capacity of the lung for carbon monoxide divided by alveolar volume; DLCO: diffusing capacity of the lung for carbon monoxide; FEV1: forced expiratory volume in the 1 second; FVC: forced vital capacity; TLC: total lung capacity.

Results

TBLB was performed using cryoprobes on 10 patients with diffuse interstitial lung diseases, whose functional and anthropometric characteristics can be seen in table 1. Between 2 and 4 transbronchial lung biopsies were obtained from each patient using a cryoprobe under fluoroscopic control, and in every case bronchoalveolar lavage was performed prior to taking the biopsy. The average duration (± standard deviation) of the procedure was 35 ± 11 min. All the patients were extubated and discharged to home. The average stay until home discharge was 156 ± 40 min. No patients had to be readmitted.

Data are shown as mean ± standard deviation, or the number of patients in the case of the sex. DLCO/AV: diffusion capacity for carbon monoxide adjusted for alveolar volume; DLCO: diffusion capacity for carbon monoxide; FEV₁: forced expiratory volume in first second; FVC: forced vital capacity; TLC: Total lung capacity.

Regarding immediate complications recorded during the procedure, 20% of the patients had grade 1 bleeding, while in 40% suffered grade 2. No patients suffered severe bleeding which made it necessary to stop the procedure or needed additional medical or surgical treatment. There were no cases of pneumothorax or acute respiratory failure after performing TBLB using cryoprobes. No other complications were evidenced 24 h after the procedure.

Twenty samples from the 10 patients (2 per patient) included in the study were analyzed by an anatomical pathologist following the established protocol. The mean area of the biopsies was 9.5mm² (range: 3-25mm²) and the mean diameter 3.1mm (range: 2.2-5mm). The mean number of well-preserved alveolar spaces was 29.6 (range: 3-100). According to the scale used for their histological examination, 60% of the samples had ≥ 75% artefact-free lung parenchyma, 50% had between 75 and 100% of viable parenchyma, and in only 2 cases a small amount of lung parenchyma was obtained, the rest of the sample being bronchial wall. As for the histological diagnoses, there were 2 cases with findings indicative of non-specific interstitial pneumonia, 3 cases of extrinsic allergic alveolitis, and in 5 cases it was not possible to make a definite histological diagnosis as the changes were non-specific.

Discussion

The results obtained make it possible to state that the TBLB with cryoprobes is a feasible technique for obtaining samples of lung parenchyma. There are relatively few complications and no apparent increases in adverse effects associated with this procedure. At present, cryoprobes have a greater freezing capacity and speed, characteristics which increase the traction of the probe on the tissue. These modifications have made it possible to increase the therapeutic efficiency of this procedure for extracting endobronchial tumours,



Figure 2. Photographs of one of the samples obtained by transbronchial lung biopsy using a cryoprobe.

with a high success rate for bronchial excision and drainage. Furthermore, in previous studies, the anatomopathological and immunohistological analyses of the tissue samples obtained with cryoprobes showed that these biopsies had a better size and quality than those obtained with conventional biopsy forceps.⁶⁻⁸

This is the first study to perform a prospective analysis of the safety and viability of this technique in patients suspected of having interstitial lung disease.

With regard to its feasibility, our results show that, when performed in a similar way to conventional procedures, using cryoprobes does not seem to increase either the difficulty of the procedure or the time it takes to carry out.

In this respect, one limitation of our study worth pointing out is the fact that the TBLB with cryoprobes were performed in an operating theatre, resulting in an increase in both their complexity and cost. However, since one of the main aims of this study was to assess safety, and at present there is little information about this, it was necessary to perform the procedure under the safest possible conditions, optimizing patient monitoring and control in order to be able to resolve any possible problems originating from its use. However, the authors believe that, with the right experience, the procedure could be carried out in a conventional, well-equipped bronchoscopy clinic.⁹

In cases of TBLB with conventional forceps, various authors have recorded reported secondary complications, with bleeding and pneumothorax being the two most common adverse effects (ranging between 1% and 5%, respectively).¹⁰⁻¹² In our study no patients had a pneumothorax or severe bleeding. Not observing severe bleeding could be connected with the meticulous selection of our patients, excluding those with a higher risk of this occurring (coagulation factor alterations and pulmonary hypertension). However, another possible reason is the effect of the cold on haemostasis (vasoconstriction and capillary microthrombosis in the area of contact of the cryoprobe), helping to reduce the number and volume of haemorrhages.

There are few data for TBLB using cryoprobes regarding the possible adverse effects of this technique. In a recent study performed on patients with diffuse lung disease who underwent TBLB using conventional biopsy forceps and cryoprobes, 4% of patients had pneumothorax and there were no cases of severe bleeding.¹³ However, it is not possible to draw definitive conclusions about the techniques' safety since both procedures were performed on the same patient, so it was impossible to establish if the few complications observed were attributable to one or other method.

Our results suggest that it is a technically feasible technique which does not seem to increase the number of adverse effects, although more extensive studies comparing this and the conventional method are necessary in order to draw conclusions about the safety of the technique. Furthermore, despite the procedure being carried out in an operating theatre in our study, it can be performed in outpatients and therefore, as with TBLB with conventional forceps, patients do not have to be admitted to hospital.

Another difference with regards to the conventional technique is that orotracheal intubation is necessary, so the patient requires deep sedation. This makes it easier to insert and remove the videobronchoscope with the cryoprobe and the sample attached to its tip, as the size of the biopsy sample means it cannot be removed through the videobronchoscope work channel. At this point, due to the patient being sedated, it is not possible to use chest pain as a sign of pleural irritation or risk of pneumothorax,^{14,15} so fluoroscopic control is important to keep the probe 1-2cm from the visceral pleura to minimise the risk of pneumothorax. On the other hand, sedation during bronchoscopy results in improved patient comfort and tolerance.¹⁶

Obtaining an accurate histological diagnosis with conventional TBLB is limited by the small size of the biopsy sample. Curley et al¹⁷ analysed 170 biopsy samples obtained using TBLB and observed that 50% were smaller than 3mm, which resulted in there also being a

small number of alveolar spaces (less than 20 in 48% of the samples). These data, along with the presence of artefacts produced by the effect of the biopsy forceps on the tissue (crushing, intraalveolar bleeding), mean that the subsequent histological analysis is more difficult and

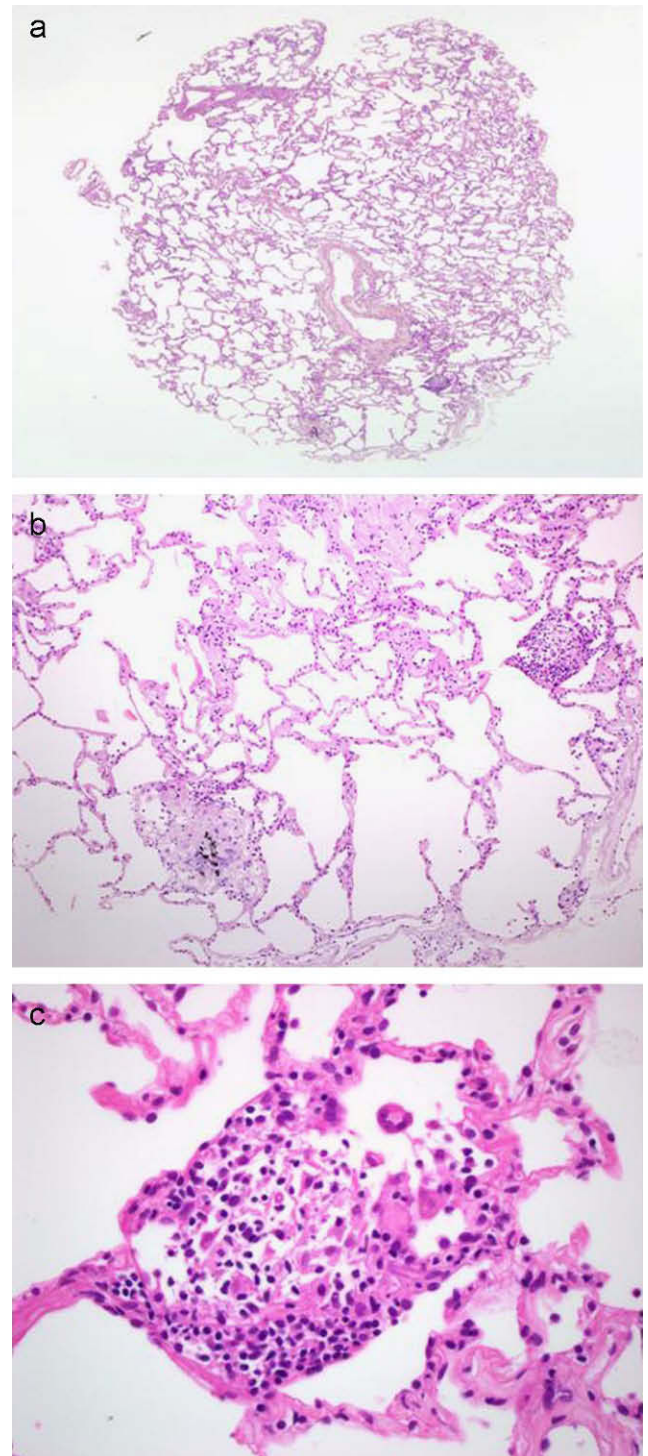


Figure 3. a) Optical microscope image ($\times 2$) of histological section of lung biopsy obtained with cryoprobe. Haematoxylin-eosin staining. Note the numerous alveolar spaces and the bronchial and alveolar structures. Patient diagnosed with extrinsic allergic alveolitis. b) Microscopic image of the same case at greater magnification ($\times 20$). Please note the well preserved alveolar structures, and the presence of mild interstitial mononuclear infiltrates and noncaseating granulomas. c) Microscopic image ($\times 40$) of a noncaseating granuloma.

that there is greater variability in the diagnostic yield with TBLB. Thus, TBLB using cryoprobes is considered an alternative method for the diagnosis of diffuse pulmonary diseases, as bigger and better quality samples are obtained with this technique than with conventional biopsy forceps (Figure 2). Microscopically, biopsy samples obtained using cryoprobes have no artefacts or secondary changes due to the effect of freezing on tissue (Figure 3). The explanation for this could be the speed with which the new generation of cryoprobes freezes the tissue. Furthermore, the alterations typically produced in the samples obtained with biopsy forceps are not evidenced.

Our clinical trial shows that using cryoprobes can improve and optimise TBLB procedures. The important prognostic and therapeutic implications make it very important to try to reach a specific diagnosis in cases of diffuse lung diseases, which are often complex and in which cooperation between the clinical doctor, radiologist and anatomical pathologist is fundamental.¹⁸ In any case, further prospective, comparative, randomised studies are necessary to see if this new method results in real increases in diagnostic yield and enables the number of surgical biopsies to be reduced.

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Conflict of Interest

The authors of this manuscript have no financial or personal relationships which could lead to a conflict of interests.

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