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ORIGINAL ARTICLE

Diagnostic yield of transbronchial cryobiopsy in interstitial lung disease: A randomized trial

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ABSTRACT

Background and objective: **Transbronchial lung biopsy (TBLB) is required for evaluation in selected patients with interstitial lung disease (ILD). The diagnostic yield of histopathologic assessment is variable and is influenced by factors such as the size of samples and the presence of crush artefacts left by conventional biopsy forceps. We compared the diagnostic yield and safety of TBLB with cryoprobe sampling versus conventional forceps sampling.**

Methods: **This randomized clinical trial analysed data for 77 patients undergoing TBLB for evaluation of ILD; patients were assigned to either a conventional-forceps group or a cryoprobe group. Two pathologists assessed the tissue samples and agreed on histopathologic diagnoses. We also compared the duration of procedures, complications and sample-quality variables.**

Results: **The most frequent diagnosis observed in the cryoprobe group was non-specific interstitial pneumonia. Histopathologic diagnoses were identified in more cases in the cryoprobe group (74.4%) than in the conventional-forceps group (34.1%) ($P < 0.001$), and the diagnostic yield was higher in the cryoprobe group (51.3% vs 29.1% in the conventional forceps group; $P = 0.038$). A larger mean area of tissue was harvested by cryoprobe (14.7 ± 11 mm^2) than by conventional forceps (3.3 ± 4.1 mm^2) ($P < 0.001$). More grade 2 bleeding (not statistically significant) occurred in the cryoprobe group (56.4%) than in the conventional-forceps group (34.2%). No differences in other complications were observed.**

Conclusions: **TBLB by cryoprobe is safe and potentially useful in the diagnosis of ILD. Larger multisite randomized trials are required to confirm the potential benefits of this procedure.**

Clinical trial registration at ClinicalTrials.gov: NCT01064609.

SUMMARY AT A GLANCE

The diagnostic yield of transbronchial lung biopsy in interstitial lung disease is variable. This is the first randomized trial to demonstrate that the yield of transbronchial lung biopsy is higher with cryoprobes than with conventional forceps in patients with interstitial lung diseases requiring a bronchoscopic procedure for diagnosis.

Key words: bronchoscopy, cryobiopsy, cryoprobe, interstitial lung disease, transbronchial lung biopsy.

Abbreviations: HRCT, high-resolution computed tomography; HSCSP, Hospital de la Santa Creu i Sant Pau; ILD, interstitial lung disease; NSIP, nonspecific interstitial pneumonia; PCNA, proliferating cell nuclear antigen; TBLB, transbronchial lung biopsy; UIP, usual interstitial pneumonia.

INTRODUCTION

Transbronchial lung biopsy (TBLB) using a flexible bronchoscope was described by Levin *et al.*[1] in 1974 and has since been widely used for obtaining lung samples for histologic evaluation.[2] The diagnostic yield of histopathologic assessment is variable,[3–5] however, and is influenced by such factors as the size of samples harvested[6] and the presence of crush artefacts left by conventional biopsy forceps.[7] Furthermore, we have found that small size and the presence of artefacts make it difficult to run immunohistochemistry assays that can be useful for quantifying fibroblasts and cytokines as markers of cell damage in the alveolar epithelium.[8,9]

Cryoprobes—used initially for therapeutic purposes—have also been useful to harvest larger samples and improve diagnostic yield in endobronchial procedures.[10] In that setting, cryobiopsies have also proven to have excellent tissue quality for

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immunohistochemistry.[11] Although the main application of cryoprobes is for the destruction of endobronchial tumours,[12] the newer generation probes freeze tissue rapidly and provide greater traction, making them attractive tools for use in diagnostic procedures. TBLB by cryoprobe seems feasible for harvesting lung parenchyma[13,14] but no randomized trials have yet confirmed the clinical utility of this procedure.

The present study was therefore designed to determine the diagnostic yield of TBLB by cryoprobe in comparison with conventional forceps in patients with clinical and radiographic findings of interstitial lung disease (ILD). We also compared the quality of the samples for histologic analysis and the safety of the two techniques. In the cryoprobe group, we explored the viability of samples for immunohistochemical assays.

METHODS

The study was conducted in the respiratory medicine department of Hospital de la Santa Creu i Sant Pau (HSCSP) in Barcelona. The stipulations of the Declaration of Helsinki for human research in clinical trials were followed, and the study was reviewed and approved by the Clinical Research Ethics Committee of HSCSP (approval number: EC/08/086/2764 (ps)). The study was registered at ClinicalTrials .gov (identifier number NCT01064609).

Study design

This randomized controlled trial of two transbronchial biopsy techniques enrolled patients who had clinical and radiographic features of ILD (either acute or chronic) and who were scheduled for TBLB in accordance with the British Thoracic Society guidelines.[15] Patients with images typical of usual interstitial pneumonia (UIP) and honeycombing pattern in high-resolution computed tomography (HRCT) were excluded, according to the same guidelines. Other exclusion criteria were use of anticoagulation therapy or presence of a coagulation disorder (thrombocytopenia < 50 000 cells/mm^3 or abnormal platelet counts >1 million cells/mm^3 international normalized ratio > 1.5, and activated partial thromboplastin time >50 s), hypoxaemia (pO_2 < 60 mm Hg), severe respiratory impairment (forced expiratory volume in 1 s < 50%, total lung capacity < 50%, diffusing capacity of carbon monoxide < 50% of reference) and unstable heart disease (uncontrolled cardiac arrhythmia, active myocardial ischaemia).

Clinical protocols

Patients were randomized to two groups to undergo TBLB with either conventional forceps or a cryoprobe by means of sealed, numbered envelopes with group assignments prepared by the department of clinical epidemiology of HSCSP. SPSS software was used to generate the randomized numbers.

In the conventional-forceps group, we used biopsy forceps (model 1556, Boston Scientific, Natick, MA, USA or FB-1556E, Olympus Medical Systems Corp, Tokyo, Japan). In the cryoprobe group, we used a flexible cryoprobe with a diameter of 2.4 mm and a length of 900 mm (model 20416-032, Erbokryo CA, Erbe, Germany).

The upper airway was anaesthetized with topical 2% lidocaine; intravenous sedation was provided throughout the procedure with midazolam in the conventional-forceps group or midazolam, remifentanil and propofol in the cryoprobe group. The endoscopic procedures were managed similarly in both groups. The only between-group procedural difference was the insertion of an endotracheal tube (Bronchoflex 7.5 mm, Rüsch, Teleflex Medical, Durham, NC, USA) in the cryoprobe group. This tube enabled us to remove the bronchoscope and cryoprobe together, along with the sampled tissue, using a technique that has been described elsewhere.[11,13,14] The patients in the cryoprobe group therefore required deeper sedation, although they continued to breathe spontaneously.

All samples were harvested through a flexible bronchoscope (BF 260-T, Olympus) during procedures that included endoscopic exploration and other diagnostic tests as required. In all patients in both groups, at least three samples were taken from several bronchial segments and lobes in affected parts of the lung that had previously been identified by HRCT. All bronchoscopies were performed under fluoroscopic guidance to help lung segment selection. In the test group, the cryoprobe was placed into the bronchial segment and applied for 3–4 s.

Procedure variables included duration of the biopsy procedure and complications (bleeding and pneumothorax). Severity of bleeding was classified on an adapted scale[16] (grade 0, no bleeding; grade 1, bleeding requiring suction to clear but no other endoscopic procedures; grade 2, bleeding requiring endoscopic procedures (bronchial occlusion-collapse and/or instillation of ice-cold saline); grade 3, severe bleeding not controlled endoscopically, causing haemodynamic or respiratory instability, requiring surgical interventions or admission to the intensive care unit). Patients were kept under observation for several hours after the bronchoscopic procedure in both groups. They were discharged home or to their usual place of residence if no complications occurred.

Tissue sample processing

Both the conventional and cryoprobe biopsies were fixed in 4% paraformaldehyde and paraffin embedded; 4-μm sections were processed for the staining protocol (HE, Masson's trichrome to identify collagen, and Orcein stain for elastic fibre detection). Two pathologists blinded to sampling method from two different hospitals evaluated the samples independently. Tissue quality variables recorded were tissue sample size (diameter and area), number of alveolar spaces sampled and percentage of the artefact-free tissue in each sample. Signs of atelectasis, tissue sample fragmentation and clots were considered

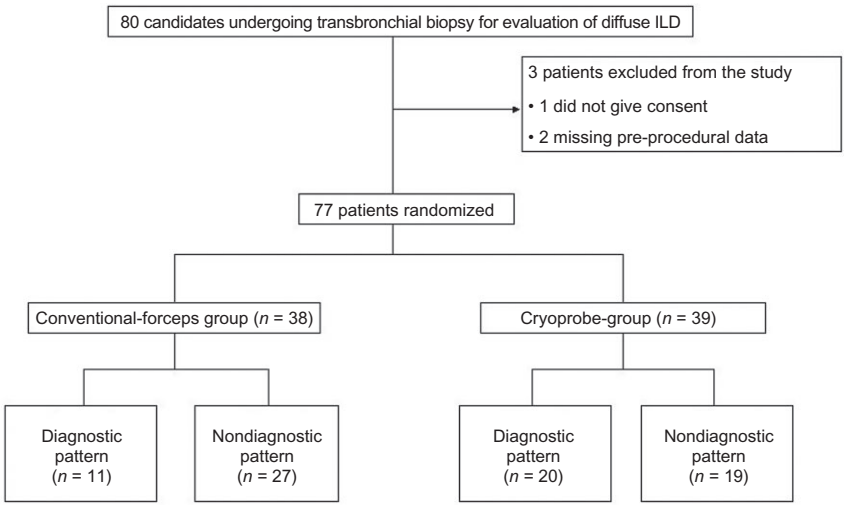

Figure 1 Flow chart of patient enrolment and findings.

artefacts; the pathologist noted them and then recorded how much of each sample was free of such artefacts. Next, the pathologists came to a consensus concerning a histologic classification of ILD.[17,18] Finally, every case was discussed in a multidisciplinary committee that included expert radiologists, clinicians and the pathologists; at this time, the samples were classified as providing a diagnostic pattern or not (i.e. having a 'nondiagnostic pattern').

Immunohistochemistry was performed on cryoprobe samples to confirm that freezing the tissue had not caused cell damage that made them nonviable for these assays. The sections were deparaffinized in xylene, ethanol and Tris-buffered saline. Membranes were stabilized in 0.2% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) and blocked with 5% normal horse serum (Vector Laboratories, Peterborough, UK). The specimens were then incubated with mouse monoclonal antibody to α-smooth muscle actin (clone 1A4; Sigma-Aldrich; working concentration, 2 μg · mL) or proliferating cell nuclear antigen (PCNA) (clone Ab-1; Calbiochem, Merk Chemicals, Darmstadt, Germany). For PCNA immunostaining, the sections were processed for high temperature epitope unmasking with commercially available equipment (2100 Antigen Retriever, Electron Microscopy Sciences, Hatfield, PA, USA) and retrieval buffer.

Statistical analysis

SPSS version 16 for Windows (IBM Corp., Armonk, NY, USA) was used for randomization and all analyses. Results are expressed as mean ± standard deviation for quantitative variables and as number and percentage for qualitative variables.

Sample size was calculated assuming an alpha error of 5%, a beta error of 20% and a 10% loss of data; we calculated that 80 patients should be enrolled to fulfil these conditions and detect at least a 32% between-method difference in the rates of histologic diagnosis.

A contingency table was constructed and inferences were tested by the chi-squared test (qualitative variables), the *t*-test (normally distributed quantitative variables) or the Mann–Whitney *U*-test (non-normally distributed quantitative variables). All comparisons were two-tailed and the level of significance was 0.05.

Table 1 Patient characteristics at baseline

Variable	Cryoprobe group (*n* = 39)	Conventional-forceps group (*n* = 38)
Age (years)	60.3 ± 10.3	64.7 ± 11.5
Gender (males/females)	20/19	16/22
FVC L	2.9 ± 0.9	2.5 ± 0.6
FVC % ref	78.2 ± 15.2	75.1 ± 17.2
FEV_1 L	2.31 ± 0.8	1.9 ± 0.5
FEV_1 % ref	82.9 ± 17.6	78.5 ± 18.6
FEV_1/FVC % ref	77.1 ± 8.5	75.9 ± 8.6
TLC % ref	88.1 ± 18.7	89.5 ± 18.9
DLco % ref	67.5 ± 19.8	64.5 ± 19.5
PaO_2 mm Hg	83.4 ± 12	78.6 ± 11.9
INR	1 ± 0.7	1.2 ± 0.5
Platelets $\times 10^9$ cells · L	249.5 ± 59.4	258 ± 98

Data are presented as mean ± SD except for gender.

% ref, percentage of reference value; DLco, carbon monoxide diffusing capacity of the lung; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; INR, international normalized ratio; PaO_2, partial pressure of oxygen in arterial blood; TLC, total lung capacity.

RESULTS

We recruited 80 candidates between February 2010 and January 2013; two patients were initially excluded because they did not meet all selection criteria (pre-procedural tests were omitted) and one patient did not sign the consent form (Fig. 1). A total of 77 patients were randomized, 38 to the conventional-forceps group and 39 to the cryoprobe group. The baseline characteristics of each group were similar (Table 1).

Table 2 Histopathologic diagnosis and diagnostic yield after multidisciplinary review

Histopathologic diagnosis				Multidisciplinary diagnosis			
Histopathologic diagnosis	Cryoprobe group *n* (%)	Conventional-forceps group *n* (%)	*P*-value	Diagnostic consensus	Cryoprobe group *n* (%)	Conventional-forceps group *n* (%)	*P*-value
• Nonspecific interstitial pneumonia	12 (30.8)	1 (2.6)		• Nonspecific interstitial pneumonia	10 (25.7)	0	
• Diffuse alveolar damage	1 (2.6)	2 (5.3)		• Acute alveolar injury	1 (2.6)	0	
• Organizing pneumonia	3 (7.7)	3 (8)		• Infection	0	2 (5.3)	
• Sarcoidosis	1 (2.6)	2 (5.1)		• Organizing pneumonia	3 (7.7)	3 (8)	
• Bronchiolitis-associated DILD	2 (5.1)	1 (2.6)		• Sarcoidosis	1 (2.6)	2 (5.3)	
• Hypersensitivity pneumonitis	3 (7.7)	0		• Respiratory-bronchiolitis associated DILD	2 (5.1)	1 (2.6)	
• Eosinophilic pneumonia	0	2 (5.3)		• Hypersensitivity pneumonitis	3 (7.7)	0	
• Adenocarcinoma	0	1 (2.6)		• Eosinophilic pneumonia	0	2 (5.3)	
• Usual interstitial pneumonia	7 (17.9)	1 (2.6)		• Adenocarcinoma	0	1 (2.6)	
Total	29 (74.4)	13 (34.1)	<0.001	Total	20 (51.4)	11 (29.1)	0.038

Data are presented as number of subjects and percentage.
DILD, diffuse interstitial lung disease.

A total of 266 lung tissue samples were harvested (126 with conventional forceps and 140 with the cryoprobe) and evaluated. The between-group difference in number of samples was not significant.

The mean duration of procedures was also similar (30.5 ± 7.6 min in the cryoprobe group and 32.5 ± 8.6 min in the conventional-forceps group, $P = 0.294$).

Diagnostic yield

The histopathologic diagnoses for all patients are shown in Table 2, by group. More samples that provided a histologic diagnosis were submitted for cryoprobe group patients (29 of 39; 74.4%) than for conventional-forceps group patients (13 of 38; 34.1%) ($P < 0.001$). Table 2 also shows the final diagnoses reached by the multidisciplinary committee after review of histologic, clinical and radiologic features. The diagnostic yield in the cryoprobe group was higher than in the conventional-forceps group (51.4% vs 29.1%, respectively) ($P = 0.038$).

Inadequate sample material for histology (<1 mm) was obtained from 24.3% of patients in the conventional-forceps group but from none in the cryoprobe group ($P = 0.001$).

Secondary outcome measures: sample quality and procedure feasibility

Tissue samples were significantly larger in the cryoprobe group than in the conventional-forceps group.

Table 3 Histologic variables and quality of biopsies

Variable	Cryoprobe group	Conventional-forceps group	*P*-value
Total no. of samples	140	126	0.342
No. of samples per procedure	3.7 ± 0.9	3.5 ± 1.2	0.44
Diameter mm	4.1 ± 1.5	1.8 ± 1	<0.001
Area mm^2	14.7 ± 11	3.3 ± 4.1	<0.001
No. alveoli sampled	68.2 ± 61.2	22.0 ± 39.8	<0.001
Presence of pleural tissue	5 (3.6%)	3 (2.4%)	0.711
Presence of bronchus wall	12 (8.6%)	10 (7.9%)	0.802
75% artefact-free (% of total sampled area)	66.6	31.6	0.012

Data are presented as mean ± SD, total number (%) of samples, or percentage of total area sampled that was 75% artefact-free area.

Tissue quality assessment showed that alveolar sampling was greater and artefacts were fewer in the cryoprobe group (Table 3).

Immunohistochemical processing demonstrated that tissue architecture was well preserved in the cryobiopsies and all were viable for the immunohistochemical detection of cytoplasmic and nuclear

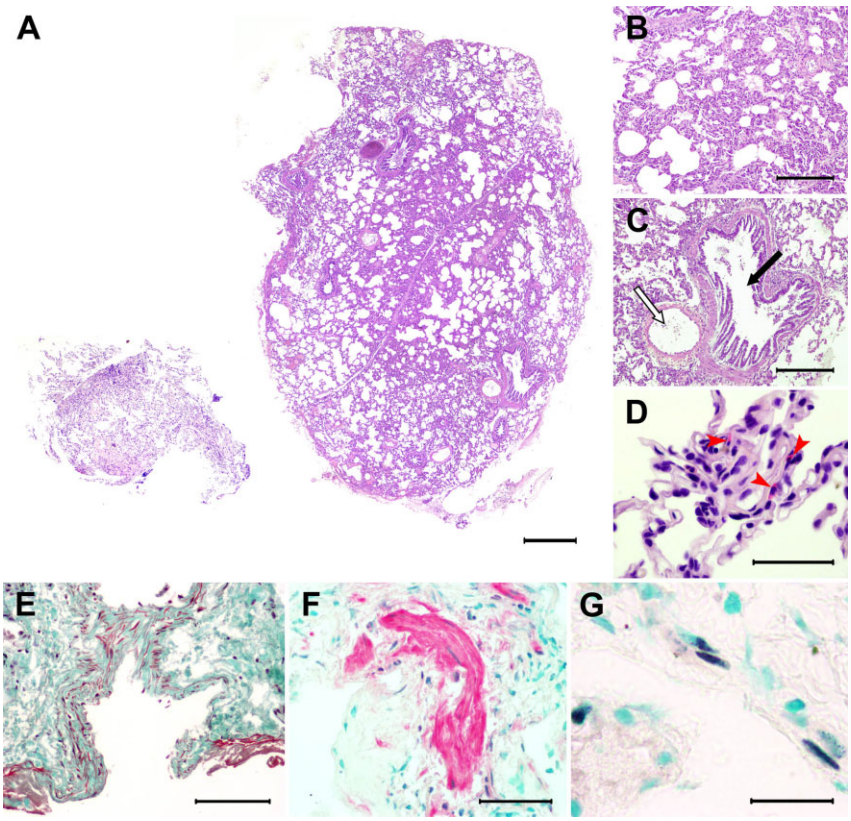

Figure 2 Four-micrometre sections were stained with HE (A-D) and Masson's trichrome (E). In (A), complete tissue sections from a conventional-forceps biopsy (left) and a cryobiopsy (right) were mapped through consecutive microscopic fields and reconstructed. The cryobiopsy preserved the lung parenchyma structure and allowed us to examine a large sampling of alveoli (A,B). Small airways (C, black arrow) and accompanying vessels (white arrow) were also sampled and preserved, and fine histopathologic details, such as infiltrating eosinophils in the parenchyma, were readily visible (D, arrows). Masson's trichrome revealed extensive extracellular matrix deposition (E). Immunostaining provided clear definition of cells positive for α-smooth muscle actin cells comprising myocytes and myofibroblasts (F, red staining) and PCNA (G, dark-purple staining). Nuclear counterstain in (F) and (G) is methyl green. Scale bars: 500 μm in (A); 200 μm in (B,C); 50 μm in (D,F); 100 μm in (E); 20 μm in (G).

Table 4 Complications during transbronchial lung biopsy

Complications	Cryoprobe group *n* (%)	Conventional-forceps group *n* (%)	*P*-value
Bleeding			0.068
Grade 0	5 (12.8)	8 (21.1)	
Grade 1	12 (30.8)	17 (44.7)	
Grade 2	22 (56.4)	13 (34.2)	
Grade 3	0 (0)	0 (0)	
Pneumothorax	3 (7.7)	2 (5.2)	0.999

Data are presented as number (%).

antigens (α-smooth muscle actin and PCNA, respectively) (Fig. 2). No freeze artefacts were observed, nor were technical difficulties experienced.

Complications

The complication rates in the two groups did not differ significantly (Table 4). Overall, a trend towards fewer cases of grade 2 bleeding was seen in the conventional-forceps group (34.2% vs 56.4% in the cryoprobe group), but the difference was not statistically or clinically significant. Two patients in the conventional-forceps group and three in the cryoprobe group developed pneumothorax. The complications were unrelated to the number of biopsies harvested and did not prolong the procedures in either group; nor were additional endoscopic procedures or surgical repair required to control bleeding in either group. All differences were nonsignificant.

Five patients (two patients in the conventional-forceps group and three in the cryoprobe group) required hospitalization for pneumothorax.

DISCUSSION

Diffuse lung disease is a common indication for TBLB. This trial, designed to compare the diagnostic yields of two TBLB procedures, demonstrates that a cryoprobe is useful for sampling the lung for ILD diagnosis in the types of patients considered candidates for this procedure according to current guidelines.[15]

In our study, the most frequent histologic diagnosis observed in the cryoprobe group was nonspecific interstitial pneumonia (NSIP). The higher diagnostic yield of cryobiopsy we observed is attributable to the larger size of the samples harvested and their higher quality in comparison with samples removed with conventional forceps. The small size of conventional samples, which contain few alveolar spaces, is an observation that has been reported by other authors, who have also remarked on the difficulty of interpreting the histologic features observed when artefacts

are present.[7,19] Although cryoprobes might be expected to cause freeze artefacts in tissue, we found higher percentages of artefact-free tissue in cryoprobe samples than in conventional-forceps samples, consistent with previous reports.[10,11] We also emphasize that cryobiopsy and subsequent fixation of the samples for immunohistochemistry gave excellent results in this study: we recorded high-quality detection of both cytoplasmic and nuclear antigens, including PCNA, a difficult epitope to unmask. The utility of immunohistochemical data available from these samples suggests new possibilities for TBLB cryoprobe-harvested samples in both research and clinical practice.

Although TBLB has an established place in the diagnosis of ILD in certain circumstances,[15] the yield of this technique when performed with conventional forceps has come under question in the context of some diffuse lung diseases.[20] The yield of TBLB has been higher when central or centrilobular involvement has been found radiologically and lower in predominantly peripheral processes such as UIP.[18] Joint American–European guidelines[21] recommend surgical lung biopsy as the technique of first choice for diagnosis of idiopathic pulmonary fibrosis, and patients with images typical of UIP and honeycombing pattern in HRCT were therefore excluded from our study. A confident diagnosis of NSIP is also sometimes based on a surgical biopsy,[15] particularly in the fibrotic subgroup. Consistent with guidelines, we observed the characteristic histopathologic pattern of chronic inflammatory infiltrates (cellular) more often in the cryoprobe group. However, interestingly, the multidisciplinary committee was able to establish the diagnosis of NSIP based on histopathologic features in cryoprobe-biopsied patients in combination with the clinical and HRCT features. When the fibrotic pattern was identified at biopsy, however, the multidisciplinary team was still unable to reach a diagnosis. Although some diagnoses could be ruled out in these cases, it was necessary to perform surgical biopsy.

The complications previously described for TBLB with forceps are severe bleeding and pneumothorax, which have been reported to occur in about 1% and 5%, respectively, of previous series.[22–24] Few studies have evaluated complications of TBLB by cryoprobe. We recently reported that no cases of pneumothorax or severe bleeding occurred in a study of 10 patients.[14]

The safety profile was similar with both tools in the present study, in which no serious complications occurred in either group. More common grade 2 bleeding in the cryoprobe group was not clinically or statistically significant, and it did not result in the harvesting of fewer samples. All bleeding could be controlled with the usual endoscopic manoeuvres (suction or occlusion). Our observations are consistent with those of authors who have used cryoprobes for removing endobronchial lesions.[25] Recently, other teams reported using a cryoprobe to safely obtain samples after lung transplantation; significantly, larger specimens were harvested with this method than with forceps.[26,27] However, those teams performed the procedures with each of the tools in the same patients and they were therefore unable to attribute the complications to a specific technique. Finally, it is also noteworthy that most of the procedures in our study could be completed within the context of our conventional outpatient bronchology service: hospitalization was only required for the five patients who developed pneumothorax.

Our study has certain limitations. First, because we randomized the patients to one procedure or the other, we were unable to compare histologic diagnosis by technique patient by patient; however, this design did allow us to establish the safety of the technique and identify complications in each group. Second, immunohistochemistry was only performed on cryoprobe-harvested samples. The reason for this decision was that our aim was to demonstrate that these rapidly frozen samples would be useful for immunohistochemistry and would not contain damaged alveoli due to freezing. Third, the number of biopsies taken is a limitation, but published studies vary as to the number of samples harvested and the optimal number for diagnosing ILD has not been established. One group concluded that diagnostic yield does not increase significantly after the third sample,[19] but others have recommended that at least five or six specimens should be taken.[28] We took at least three samples (range, three to six) from several bronchial segments in the affected parts of the lung previously identified by HRCT. Finally, although the study was randomized in design and the pathologists were formally blinded as to sampling technique, the blinding could have been ineffective given the size differences in samples. However, they were required to record tissue quality variables according to an objective, pre-established protocol.

In summary, our results confirm that cryoprobe TBLB is safe and potentially useful in the diagnosis of ILD in patients selected according to current guidelines.[15] Histologic diagnostic yield is higher when the cryoprobe is used, attributable to the larger, higher-quality samples harvested although, larger multicentre randomized trials of TBLB cryobiopsy are required to confirm the benefits and safety profile we observed for this technique. Multidisciplinary cooperation between clinicians, radiologists and pathologists remains essential for analysing the findings of TBLB procedures.

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Society for Pulmonology (SOCAP 2008), and the Spanish Association of Respiratory Endoscopy (AEER 2008).

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