Eur Respir J 2012; 39: 685–690
DOI: 10.1183/09031936.00033011

Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial

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ABSTRACT: Forceps, brushes or needles are currently the standard tools used during flexible bronchoscopy when diagnosing endobronchial malignancies. The new biopsy technique of cryobiopsy appears to provide better diagnostic samples. The aim of this study was to evaluate cryobiopsy over conventional endobronchial sampling.

A total of 600 patients in eight centres with suspected endobronchial tumours were included in a prospective, randomised, single-blinded multicentre study. Patients were randomised to either sampling using forceps or the cryoprobe. After obtaining biopsy samples, a blinded histological evaluation was performed. According to the definitive clinical diagnosis, the diagnostic yield for malignancy was evaluated by a Chi-squared test.

A total of 593 patients were randomised, of whom 563 had a final diagnosis of cancer. 281 patients were randomised to receive endobronchial biopsies using forceps and 282 had biopsies performed using a flexible cryoprobe. A definitive diagnosis was achieved in 85.1% of patients randomised to conventional forceps biopsy and 95.0% of patients who underwent cryobiopsy ($p<0.001$). Importantly, there was no difference in the incidence of significant bleeding.

Endobronchial cryobiopsy is a safe technique with superior diagnostic yield in comparison with conventional forceps biopsy.

KEYWORDS: Cryobiopsy, diagnostic yield, endobronchial biopsy, forceps, multicentre, tumour

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Received:
Feb 21 2011
Accepted after revision:
July 08 2011
First published online:
Aug 18 2011

Flexible bronchoscopy is the diagnostic tool of choice to diagnose endobronchial malignancies. It allows inspection and biopsy of any endobronchial abnormalities under direct vision. Pathology samples can be harvested by using various techniques, *e.g.* forceps, brushing or washing. Flexible bronchoscopy and the associated tissue sampling techniques are the most widespread procedures in the diagnosis of central lung cancer. Even though the specimens are obtained under direct vision, there is a significant failure rate, which, therefore, requires repeated bronchoscopies. Concurrent application of different sampling techniques at bronchoscopy has been shown to improve the yield [1–3].

The major drawback of the forceps biopsy technique is the relatively small amount of tissue obtained, which is determined by the size of the forceps. Additionally, mechanical compression or crush artefacts from the instrument tip cause alterations of the tissue samples, which affect the quality of the histological analysis [4].

A biopsy tool of choice should provide a safe technique that is capable of obtaining large biopsy samples without causing any morphological alteration to the tissue samples, thereby lowering the rate of additional sampling techniques needed, or even the need for repeated bronchoscopies. It should also enable sampling from areas of the endobronchial tree that may be difficult to access. The flexible cryoprobe appears to have most of these characteristics.

Successful removal of endobronchial tumour from the central airways by using flexible cryoprobes has been previously demonstrated [4, 5]. Tissue samples from cryorecanalisation were demonstrated to be of a high quality and much larger than conventional biopsy samples [4].

The aim of this multicentre study was to evaluate the diagnostic yield and safety of cryobiopsy in

For editorial comments see page 513.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

comparison with conventional forceps biopsy for sampling endobronchial lesions suspicious for malignancy.

MATERIALS AND METHODS

This study was a prospective, randomised, single-blinded, controlled, multicentre study. The primary goal of the study was to assess the diagnostic yield of cryobiopsies in comparison with forceps biopsies. The gold standard was the final pathological diagnosis and takes into consideration any other diagnostic investigations that were performed. Secondary endpoints of the study were to assess the duration of the biopsy procedure, the number of samples taken, the level of difficulty in positioning the probe and the amount of bleeding.

Inclusion criteria were: suspected endobronchial lesion based on clinical signs and radiological images; age >18 yrs; and signed informed consent.

Patients with a bleeding diathesis or who were on anticoagulants or who had oxygen saturation <90% (under delivery of oxygen at $\leqslant 2$ L·min^{-1}), or severe underlying cardiac disease (unstable angina pectoris, myocardial infarction in the past month or decompensated heart failure) were excluded.

The corresponding ethics committees and the institutional review board at the University of Ulm, Ulm, Germany, approved the study protocol.

Bronchoscopy

Written informed consent for participation in the study and for data protection was obtained before bronchoscopy. The protocol allowed the bronchoscopy to be performed either by the flexible or rigid technique. However, all patients who underwent flexible bronchoscopy were required to be intubated with an endotracheal tube in order to provide a secure airway and also to enable cryobiopsies to be performed. Where rigid bronchoscopy was utilised, the actual biopsy was performed using the flexible bronchoscope inserted through the rigid tube. General anaesthesia for rigid bronchoscopy, as well as sedation for flexible bronchoscopy, was performed according to each centre's standards. Standard patient monitoring comprised continuous O_2 saturation and ECG monitoring with repeated blood pressure measurements.

The patients were randomised only after a suspicious lesion requiring a biopsy was identified at bronchoscopy, providing they had been enrolled into the study and had signed a consent form. A stratified block randomisation into forceps or cryobiopsy group was performed, giving the information by using consecutive numbered envelopes for randomisation at each study site.

Tissue sampling

Depending on randomisation, either cryobiopsy or forceps biopsy was performed. With cryobiopsy, the cryoprobe was placed onto the suspicious lesions, and the freezing cycle was then initiated causing the tissue to attach to the probe tip [4–6]. The duration of the freezing cycle was dependent on the tissue characteristics and was judged by the operator according to the size of the frozen tissue formed. Freezing for ~2–3 s was considered as sufficient for most of the biopsies. Whilst still frozen, the cryoprobe was retracted together with the bronchoscope to extract a biopsy sample. The frozen biopsy was then released from the probe by thawing in a water bath and placed in formalin.

The number of biopsies needed per protocol was left to the bronchoscopist's discretion with a suggested maximum limit of four samples. The actual number of biopsies and their localisation were documented, as well as any significant bleeding or other complications. Tumour growth was classified into exophytic or submucosal. Duration of the biopsy procedure, and type and amount of medication were documented. Additionally, the difficulty of positioning the biopsy instrument on the lesion was rated to be easy, moderate or difficult.

Histology

The biopsy samples were fixed in neutral 10% buffered formalin, embedded in paraffin and cut into 4-μm sections followed by staining with haematoxylin and eosin. The samples were analysed and assessed by one pathologist in each centre according to common standards describing malignancy and its entity. The pathologist was blinded from the biopsy technique that had been used. To allow an exact classification, additional stainings and immunohistochemistry were allowed.

Statistics

All data were analysed by descriptive methods. For categorical variables, absolute and relative frequencies, and for continuous variables mean$\pm$SD, are given, whereby the primary and secondary end-points are presented separately for both groups.

The biopsy technique utilised was regarded as successful when histological confirmation of the diagnosis was achieved at the initial bronchoscopy and matched the final diagnosis. If additional tests, *e.g.* further bronchoscopies, surgery, *etc.*, were needed to establish the diagnosis, the biopsy was regarded as nondiagnostic. Diagnostic yield was calculated for each biopsy technique as the number of diagnostic procedures divided by number of nondiagnostic procedures plus the number of diagnostic procedures. An explorative test of the diagnostic yield of the two biopsy techniques was performed in a small number of patients before statistical planning of this trial. On the basis of these data, the study was powered at 90% for a level of significance of $p=0.05$. A group size of 278 patients was calculated and, assuming possible dropouts, a group size of 300 patients was proposed.

The primary confirmatory comparison of diagnostic rate between the two techniques was evaluated by a two-tailed Chi-squared tests with a 5% level of significance.

All additional secondary assessments were investigated using the respective tests for parallel-group designed studies (Chi-squared test and Mann–Whitney rank tests). The respective levels of significance in this exploratory analysis were set to 5%, and no adjustment for multiple testing was performed.

All adverse events were recorded and compared descriptively. Bleeding was defined according to the clinical interventions required. Mild bleeding was defined as bleeding that was controlled by suctioning. Severe bleeding was regarded as the need for additional intervention, *e.g.* instillation of ice-cold saline or a diluted vasoconstrictive drug, balloon tamponade, argon plasma coagulation (APC), conversion to rigid bronchoscopy or mechanical ventilation.

RESULTS

A total of 600 patients were randomised from June 2006 to October 2008 in eight centres in Germany. 593 of these patients were included in the evaluation; five patients withdrew their written consent and two patients were randomised twice. Malignant disease was diagnosed in 563 patients (fig. 1). 388 patients had nonsmall cell lung cancer (NSCLC), 118 patients small cell lung cancer (SCLC) and 57 patients other malignant entities, including metastases (table 1 and fig. 1).

The demographic and baseline characteristics of the two groups were similar (table 1). The patients were ~65 yrs of age with a male preponderance. There was no difference in the proportion of patients that were taking aspirin between the two groups (25 *versus* 27 patients) or clopidogrel (one *versus* four patients). General anaesthesia was used in 134 patients in the forceps group and 136 patients in the cryobiopsy group. For conscious sedation, propofol was used in 174 patients, 42 patients received midazolam and 92 received a combination of both. In 15 patients, the intubation was performed exclusively under local anaesthesia. There was no difference in the rates of rigid bronchoscopy and flexible bronchoscopy (forceps group: rigid in 133 patients and flexible in 164 patients; cryobiopsy group: rigid in 136 patients and flexible in 160 patients). Different kinds of biopsy forceps were allowed according to the personal preferences of each centre or physician. These were standard biopsy forceps (with and without thorn) having claw diameters of 2.0 mm and 2.6 mm, and crocodile forceps with claw diameters of 2.0 mm and 2.6 mm. Forceps with smaller claws (2.0 mm) were used in 76 patients (25.6%) and the forceps with larger claws (2.6 mm) in 219 patients (73.7%). In two cases, specification of the forceps was not documented. The diagnostic yield was not different in patients who were diagnosed with the small forceps (2.0 mm; 84.2%) or the large forceps (2.6 mm; 85.4%).

No difference in the coagulation parameters and thrombocyte count was found. There was no significant difference in the location of the lesion between the groups ($p=0.40$; table 1).

Among patients with the diagnosis of a malignant disease, the diagnostic yield for cryobiopsy was 95.0% (268 out of 282 patients) and 85.1% (239 out of 281 patients) for standard forceps. Comparison between groups revealed a significantly higher diagnostic yield for cryobiopsy ($p<0.001$; table 2). Cryobiopsy exceeded forceps biopsy significantly in the diagnosis of both exophytic (97.3% *versus* 89.5%; $p=0.003$) and submucosal tumours (90.9% *versus* 75.8%; $p=0.005$) (table 2).

The final diagnosis could not be made with the instrument predefined by randomisation in 56 patients (42 of the forceps biopsy and 14 of the cryobiopsy patients). In these patients, the definitive diagnosis was obtained by many alternative procedures, including surgery as a final choice (table 3).

With respect to the bronchoscopy technique, the diagnostic yield using flexible bronchoscopy was significantly higher with cryobiopsy (95.2%) compared with forceps biopsy (82.2%) ($p=0.0001$). When rigid bronchoscopy was used, there was a trend towards a higher diagnostic yield in the cryobiopsy group (94.6% *versus* 89.5%; $p=0.131$).

In order to further demonstrate the added value of the cryobiopsy technique with respect to the histopathological characteristics of different tumour entities, we performed a subgroup analysis for

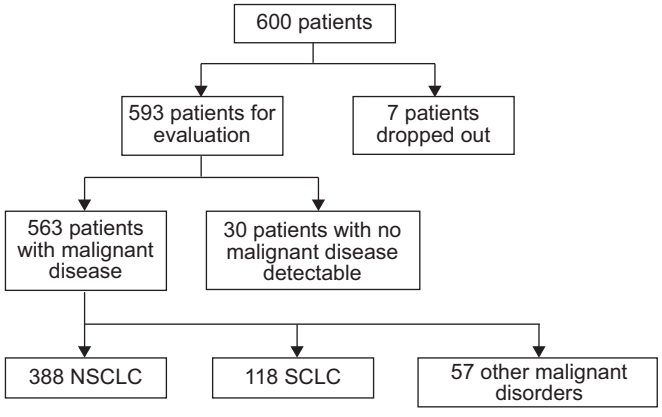

FIGURE 1. Patient distribution. NSCLC: nonsmall cell lung cancer; SCLC: small cell lung cancer.

TABLE 1 Patient characteristics and distribution of the biopsy sites for each biopsy technique

	Forceps	Cryoprobe	p-value
Patient characteristics			
Patients	297	296	
Age yrs	65.3±9.9	64.8±10.3	0.55
Male	207 (69.6)	217 (73.4)	0.36
Body height cm	170.9±8.5	170.2±8.9	0.33
Body weight kg	74.3±14.7	72.3±15.0	0.10
Aspirin 100 mg·day^{-1}	25 (8.42)	27 (9.12)	0.77
Clopidogrel 75 mg·day^{-1}	1 (0.34)	4 (1.35)	0.22
General anaesthesia	134 (45.1)	136 (45.9)	0.87
Rigid bronchoscopy	133 (44.8)	136 (45.9)	0.80
PT %	95.1±16.6	94.7±16.2	0.77
PTT s	29.8±4.6	29.5±4.6	0.43
Thrombocyte count $\times 10^3$ cells·μL^{-1}	331.6±130.9	331.0±118.8	0.95
NSCLC	192	196	0.73
SCLC	62	56	0.61
Other malignant disease	27	30	0.68
Other disease	16	14	0.85
Distribution of the biopsy sites			
Trachea	18	16	0.40
Main bronchi	62	57	
Lobe bronchi and intermediate bronchus	156	175	
Segmental bronchi	61	48	
Lesions in patients with malignancy			
Exophytic	190	183	0.49
Submucosal	91	99	

Data are presented as n, n (%) or mean±SD, unless otherwise stated. None of the values differed significantly between the two biopsy groups. PT: prothrombin time; PTT: partial thromboplastin time; NSCLC: nonsmall cell lung cancer; SCLC: small cell lung cancer.

TABLE 2 Diagnostic and nondiagnostic biopsies for each biopsy technique in patients with maligancy

	Forceps	Cryoprobe	p-value
Overall			
Diagnostic	239 (85.1)	268 (95.0)	<0.001
Nondiagnostic	42 (14.9)	14 (5.0)	
Exophytic tumour			
Diagnostic	170 (89.5)	178 (97.3)	0.003
Nondiagnostic	20 (10.5)	5 (2.7)	
Submucosal tumour			
Diagnostic	69 (75.8)	90 (90.9)	0.005
Nondiagnostic	22 (24.2)	9 (9.1)	

Data are presented as n (%), unless otherwise stated. Biopsies are shown in relation to each biopsy group and subgroup analysis of exophytic and submucosal tumours. Cryobiopsy was superior over forceps biopsy in the diagnosis of exophytic and submucosal tumours.

the two largest tumour entities in this collective: NSCLC and SCLC (table 4). The proportion of nondiagnostic results in patients with NSCLC and SCLC was lower after cryobiopsy than after forceps biopsy: NSCLC, 5.5% (95% CI 2.8–9.6) *versus* 11.8% (95% CI 7.6–17.2) and SCLC, 3.6% (95% CI 0.4–12.3) *versus* 16.1% (95% CI 8.0–27.7) (table 4). The cryobiopsy group shows a considerably smaller 95% confidence interval in comparison with the forceps group regarding the nondiagnostic outcomes of NSCLC and SCLC. This indicates that, cryobiopsy not only serves as a more sensitive but also as a more reliable tool than forceps biopsy for diagnosis.

TABLE 3 Procedures used to obtain the final malignant diagnosis after nondiagnostic forceps biopsy and cryobiopsy

	Forceps	Cryoprobe
Subjects	42	14
Type of procedure for final diagnosis		
Cryobiopsy	14	0
Forceps	14	3
Forceps + cytology	2	0
Forceps + catheter biopsy	0	1
Cervical lymph node biopsy	2	0
Catheter biopsy	0	1
Brush cytology	1	0
Cytology	2	0
Liver function	0	1
Transbronchial biopsy of a peripheral nodule	2	0
TBNA	0	2
Transcranial fine needle aspiration	1	0
Punch biopsy	0	2
Lymph node biopsy	0	1
Biopsy from stomach metastasis	0	2
Mediastinoscopy	1	0
Surgery	3	1

Data are presented as n. The procedures used to obtain the final diagnosis after nondiagnostic procedures differed in the forceps biopsy and the cryobiopsy group. There was no preferred second choice method. TBNA: transbronchial needle aspiration.

TABLE 4 Numbers of nondiagnostic biopsies for each biopsy technique

Two most common tumour types	Forceps	Cryoprobe	p-value
NSCLC			
n/N	23/195	11/201	0.025
% (95% CI)	11.8 (7.6–17.2)	5.5 (2.8–9.6)	
SCLC			
n/N	10/62	2/56	0.024
% (95% CI)	16.1 (8.0–27.7)	3.6 (0.4–12.3)	

NSCLC: nonsmall cell lung cancer; SCLC: small cell lung cancer.

No difference was found with respect to the time needed for biopsy and subsequent bleeding control between the groups 5.05 ± 4.54 *versus* 5.25 ± 4.20 min for cryobiopsy and forceps biopsy, respectively. The number of samples taken differed in favour of cryobiopsy (3.24 ± 1.16 *versus* 3.45 ± 0.95; $p<0.001$). Positioning judgement was not different between the groups, although there was a trend in favour of the cryobiopsy ($p=0.068$, data not shown). There was significantly more bleeding in the cryobiopsy group compared with the forceps group ($p=0.009$; table 5); however, the number of bleeding complications needing any intervention for bleeding control did not differ between the groups ($p=0.90$). APC was required in 13 patients from the cryobiopsy group compared with eight patients in the forceps group. Tamponade was required in two patients in each group. All other episodes of bleeding were managed by instillation of ice-cold saline or vasoconstrictive drugs. No surgical interventions for bleeding control were needed and no fatal events occurred.

DISCUSSION

This is the first multicentre, prospective, randomised, single-blinded, controlled trial evaluating the novel cryobiopsy technique in comparison with our current standard technique using forceps. We were able to demonstrate that a greater proportion of patients with an endobronchial lesion suspicious for malignancy has a definite diagnosis when a cryobiopsy is performed (95%)

TABLE 5 Biopsy-related bleeding for each biopsy group

Type of bleeding	Forceps	Cryoprobe	p-value
None	91 (30.6)	59 (19.9)	0.009
Mild[#]	153 (51.5)	183 (61.8)	
Severe[¶]	53 (17.8)	54 (18.2)	

Data are presented as n (%), unless otherwise stated. [#]: no intervention; [¶]: at least one intervention for bleeding control applied.

rather than a traditional forceps biopsy (85.1%). With our current standard practice of forceps biopsy, the patients without a conclusive diagnosis (15%) would have required either a repeat bronchoscopy or an alternative procedure. This has both cost implications and the need for the additional invasive procedures, which increase the risk of adverse events in any individual patient. This statement may be limited due to the secured airway also for flexible bronchoscopy, demanded by the protocol. Furthermore, the need for additional procedures would increase any potential time delays to the patient's treatment. The diagnostic rate for cryobiopsy is the highest rate observed for any single sampling technique in bronchoscopy [7–9]. Even where multiple sampling techniques (forceps, needles, brushing and washing) are utilised, the diagnostic yield reaches only 88% [2, 8, 10, 11]. The difference in our results is unlikely to be due to a poor yield in the standard forceps group, as its diagnostic rate is similar to other published studies [7–9, 10]. However, superiority of cryobiopsy becomes blurred when exclusively comparing biopsies carried out using rigid intubation. Due to general anaesthesia and a reduced breathing amplitude, positioning of forceps becomes easier, thus diminishing the benefit of cryobiopsy. The overall superiority of cryobiopsies is most probably due to the higher quality of the samples defined by their larger sample size and low amount of biopsy-related tissue alterations, which has been shown in previous studies [4, 6, 12, 13]. Most recently, a single-centre analysis underlined this fact: the total area of each tissue section of the cryobiopsies had been described to be twice as large as forceps biopsies, in addition to the higher quality of the cryobiopsies. This resulted in a significantly higher artefact-free area of each slide in the cryobiopsy group compared with the forceps group (9.6 *versus* 3.6 mm^2). This is of increasing importance in the treatment of lung cancer. Increasingly, drugs that target specific genetic alterations in the tumour tissue are being utilised [14, 15] and, hence, a better quality of tissue obtained at biopsy might facilitate identification of molecular targets for treatment.

Further advantages of the cryoprobe include the fact that biopsies can be extracted even when the cryoprobe is positioned tangentially with an angle of 0° towards the tissue, whereas the forceps must be placed almost perpendicular to the tissue to obtain a good specimen. This is an important advantage, especially in a narrower lumen. Due to a concentric expansion of the freezing area starting from the tip of the cryoprobe and expanding into the periphery, a larger surface area and, thereby, larger biopsies can be generated. The size can also be regulated by the operator over the activation time: increasing the freezing time increases the biopsy size. Forceps biopsies are limited by the size of the forceps claws. These technical differences may also account for the significantly higher number of diagnostic biopsies, mostly in submucosal (90.9% *versus* 75.8%; p=0.005) but also in exophytic tumours (97.3% versus 89.5%; p=0.003).

In addition, cryobiopsy could serve as a more sensitive and more reliable tool than forceps biopsy to diagnose both NSCLC and SCLC. We found that cryobiopsy was superior to forceps biopsy in the diagnosis of NSCLC (p=0.025) and SCLC (p=0.024) (table 4).

One histological characteristic of SCLC is the large amount of necrotic tissue present. As the size of the cryobiopsy specimen is larger, this may affect the diagnostic yield of cryobiopsy less negatively than with forceps biopsy. A more detailed histological evaluation, especially with respect to NSCLC and SCLC subtyping, will be the subject of further studies.

The safety profile for the two techniques was similar. The adverse events, particularly bleeding, were quoted on the basis of interventions required. This is far more clinically relevant than attempting to estimate the volume loss of the blood, which is notoriously difficult. There was a greater incidence of mild bleeding with cryobiopsy, but no additional interventions were required. This observation might be explained by the fact that, after retracting the bronchoscope for harvesting the biopsy, there is a larger time period until bleeding control can be started, resulting in an accumulation of blood at the biopsy site. This assumption is in line with the data of an animal study, where no difference in bleeding times was observed between forceps biopsy and cryobiopsy, although larger biopsies were extracted with the cryoprobe [16].

One limitation that might be argued is that only malignant disease was included for calculation, for several reasons. First, the degree of separation between a diagnostic and nondiagnostic result is stronger than in benign disease. Secondly, in the setting of a multicentre study, it was important to have a standardised diagnostic process, which is defined best for malignancies.

A disadvantage of the cryobiopsy technique is that intubation of the patients is recommended. The tissue attached to the cryoprobe cannot be retracted through the instrument channel of the bronchoscope and, hence, requires removal of the cryoprobe and bronchoscope as a single unit. Under these circumstances, it is important to have a secure airway allowing rapid re-insertion of the bronchoscope to control any potential bleeding. This also facilitates additional suction if required and insertion of tamponade balloons if needed. However, we consider this to be a small additional step and most patients can tolerate an endotracheal tube without the need for additional sedation in comparison with a standard bronchoscopy. Our results clearly demonstrate that there is no difference in the duration of the two procedures, or in the sedation or anaesthetic protocols. Furthermore, the procedure can be performed according to local practice with either flexible bronchoscopy or in conjunction with rigid bronchoscopy.

In conclusion, endobronchial cryobiopsy is a safe technique with a higher diagnostic yield for the diagnosis of endobronchial malignancies than forceps biopsy and might extend the number of tools available to chest physicians for obtaining sufficient endobronchial tissue for a definitive diagnosis.

CLINICAL TRIAL

This study is registered at www.controlled-trials.com with identifuer number ISRCTN97376650.

STATEMENT OF INTEREST

Statements of interest for J. Hetzel, M.N. Szyrach, P.L. Shah and M. Hetzel can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

ACKNOWLEDGEMENTS

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