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

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Abstract

Objective

Currently the use of forceps, brushes or needles during flexible bronchoscopy are the standard tools 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.

Materials and Methods

A total of 600 patients in 8 centres with suspected endobronchial tumours were included in a prospective, randomized, single blinded multi-centre study. Patients were randomized either to 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-square test.

Results

A total of 593 patients were randomised, of which 563 had a final diagnosis of cancer. 281 patients were randomized to endobronchial biopsies using forceps and 282 had biopsies performed using a flexible cryoprobe. A definitive diagnosis was achieved in 85.1% of patients randomized 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.

Conclusions

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

Keywords

Cryobiopsy, endobronchial biopsy, forceps, multicentre, diagnostic yield, tumor

Introduction

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, 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 requiring repeated bronchoscopies. Concurrent application of different sampling techniques at bronchoscopy has been shown to improve the yield (1),(2),(3).

The major drawback on forceps biopsy techniques is a relatively small amount of tissue, determined by the size of the forceps. Mechanical compression or crush artefacts from the instrument's tip causes alterations of the tissue samples, which affects the quality of the histological analysis (4).

A biopsy tool of choice should combine the following characteristics: safe technique, which is capable of obtaining large biopsy samples without causing any morphological alteration to the tissue samples. Thus it lowers the rate of additional sampling techniques needed or even 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

cryorecanalization were demonstrated to be of a high quality and much larger than conventional biopsy samples (4).

The aim of this multi-centre study was to evaluate the diagnostic yield and safety of cryobiopsy in comparison to conventional forceps biopsy for sampling endobronchial lesions suspicious for malignancy.

Materials and Methods

This study was a prospective, randomized, single blinded, controlled, multicentre study. The primary goal of the study was to assess the diagnostic yield of cryobiopsies in comparison to 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 to position the probe and the amount of bleeding.

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

Patients with a bleeding diathesis or on anticoagulants, oxygen saturation below 90% (under delivery of oxygen at up to 2l/min), 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 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 utilized, 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 allowed to be performed according to each centres' standards. Standard patient monitoring comprised continuous O_2 saturation and ECG monitoring with repeated blood pressure measurements.

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

Tissue Sampling

Depending on randomization either cryobiopsy or forceps biopsy was done. With cryobiopsy the cryoprobe was placed onto the suspicious lesions, and then the freezing cycle was initiated causing the tissue to attach onto the probe's tip (4),(5),(6). The duration of the freezing cycle was dependant on the tissue characteristics and was

judged by the operator according to the size of the frozen tissue formed. Freezing for approximately 2-3 seconds 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 then placed in formalin.

Per protocol, the number of biopsies needed was left to the bronchoscopist's discretion with a suggested maximum limit of four samples. The actual number of biopsies and their localization were documented as well as any significant bleeding or other complications. Tumour growth was classified into exophytic or submucosal. Duration of the biopsy procedure, type and amount of medications were documented. Additionally, the difficulty of the biopsy instrument being positioned 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 in 4 µm sections followed by staining with haematoxylin and eosin. The samples were analyzed and assessed by one pathologist in each centre according to common standards describing malignancy and its entity. The pathologist was blinded for the biopsy technique~~,~~ which was 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 and standard deviation were given, whereby the primary and secondary endpoints are presented separately for both groups.

The biopsy technique utilized 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 non-diagnostic. Diagnostic yield was calculated for each biopsy technique as number of diagnostic procedueres diveded by number of non-diagnostic procedures plus number of diagnostic procedures. An explorative test on the diagnostic yield of the two biopsy techniques was done 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 0.05. A group size of 278 patients was calculated - assuming possible drop outs 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-square tests with a 5% level of significance.

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

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, conversion to rigid bronchoscopy or mechanical ventilation.

Results

A total of 600 patients were randomized from June 2006 to October 2008 in 8 centres. Five hundred ninety-three (593) of these patients were included in the evaluation, five patients withdrew their written consent and two patients were randomized twice. Malignant disease was diagnosed in 563 patients (figure 1). 388 patients had NSCLC, 118 patients SCLC and 57 patients other malignant entities including metastases (table 1 and figure 1).

The demographic and baseline characteristics of the two groups were similar (table 1). The patients were about 65 years of age with a male preponderance. There was no difference in the proportion of patients that were taking Aspirin between the two groups (25 vs. 27 patients) or clopidogrel (1 vs. 4 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, 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, flexible in 164 patients, cryobiopsy group: rigid in 136 patients, 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 2.0mm and 2.6mm and crocodile forceps with claw diameters of 2.0 mm and 2.6 mm. Forceps with smaller claws (2.0mm) were used in 76 patients (25.6%) and the forceps with larger claws (2.6mm) in 219 patients (73.7 %). In 2 cases specification of the forceps was not documented. The diagnostic yield was not different

in patients who were diagnosed with the small forceps (2.0mm: 84.2%) or the large forceps (2.6mm: 85.4%).

No difference in the coagulation parameters and the 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 tumours (97.3% vs. 89.5%, p=0.003) and submucosal tumours (90.9% vs. 75.8%, p=0.005) (see table 2).

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

With respect to the bronchoscopy technique, the diagnostic yield using flexible bronchoscopy was significantly higher with cryobiopsy (95.2%) compared to 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 the two largest tumour entities in this collective: NSCLC (Non-

small cell lung cancer) and SCLC (Small cell lung cancer) (see table 4). The proportion of non-diagnostic results in patients with NSCLC and SCLC was lower after cryobiopsy than after forceps biopsy: 5.5% (95%-CI: 2.8 – 9.6) vs 11.8% (95%-CI: 7.6 – 17.2) (NSCLC) and 3.6% (95%-CI: 0.4 – 12.3) vs 16.1% (95%-CI: 8.0 – 27.7) (SCLC) (see table 4). The cryobiopsy group shows a considerably smaller 95%-confidence interval in comparison to the forceps group regarding the non-diagnostic 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.

No difference was found with respect to the time needed for biopsy and subsequent bleeding control between the groups 5.05 ± 4.54 min vs. 5.25 ± 4.20 min for cryobiopsy and forceps biopsy, respectively). Number of samples taken differed in favor for cryobiopsy (3.24 +/– 1.16 vs. 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 significant more bleeding in the cryobiopsy group compared to 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 ($p = 0.90$). Argon plasma coagulation (APC) was required in 13 patients from the cryobiopsy group compared to 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 multicentric, prospective, randomized, single blinded, controlled trial evaluating the novel cryobiopsy technique in comparison to our current standard technique using forceps. We could demonstrate that a greater proportion of patients with an endobronchial lesion suspicious for malignancy has a definite diagnosis when a cryobiopsy is performed (95%) 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),(8),(9). Even where multiple sampling techniques (forceps, needles, brushing, washing) are utilized the diagnostic yield only reaches 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),(8, 9),(10). However superiority of cryobiopsy becomes blurred when comparing exclusively biopsies done in rigid intubation. Due to general anaesthesia and a reduced breathing amplitude positioning of forceps gets 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),(16). 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 higher quality of the cryobiopsies (13). This resulted in a significantly higher artefact-free area of each slide in the cryobiopsy group compared with the forceps group (9.6mm^2 vs. 3.6mm^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 utilized (14) (15). Hence, 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 towards 0° to the tissue whereas the forceps has to be placed almost perpendicular on the tissue to get a good specimen. This is an important advantage especially in narrower lumen. Due to a concentric expansion of the freezing area starting from the tip of the cryoprobe 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 significant higher amount of diagnostic biopsies mostly in submucosal (90.9% vs. 75.8%, p = 0.005) but also in exophytic tumours (97.3% vs 89.5%, p = 0.003).

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

One histological characteristic of SCLC is the large amount of necrotic tissue. Since 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 a matter 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 trying 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 gap 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 and cryobiopsy, although larger biopsies were extracted with the cryoprobe (16).

One limitation might be argued is, that only malignant disease was included for calculation for several reasons: first the degree of separation between diagnostic and non- diagnostic is stronger than in benign disease. Second in the setting of having multiple study centres it was important to have a standardized 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. It also facilitates additional suction if required and insertion of tamponade balloons if required. However, we consider this as a small additional step and most patients can tolerate an endotracheal tube without the need of additional sedation in comparison to a standard bronchoscopy. Our results clearly demonstrate that there is no difference in the duration of the two procedures and sedation or anaesthesia 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 chest physician's armamentarium of obtaining sufficient endobronchial tissue for a definitive diagnosis.

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

	Forceps	Cryoprobe	P value
Patient characteristics			
Number of patients	297	296	
Age [years]	65.3 ± 9.9	64.8 ± 10.3	p = 0.55
Male	207 (69.6%)	217 (73.4%)	p = 0.36
Body height [cm]	170.9 ± 8.5	170.2 ± 8.9	p = 0.33
Body weight [kg]	74.3 ± 14.7	72.3 ± 15.0	p = 0.10
Aspirin 100mg/d [patients]	25 (8.42%)	27 (9.12%)	p = 0.77
Clopidogrel 75mg/d [patients]	1 (0.34%)	4 (1.35%)	p = 0.22
General anesthesia [patients]	163 (54.9%)	160 (54.1%)	p = 0.87
Rigid bronchoscopy [patients]	164 (55.2%)	160 (54.1%)	p = 0.80
PT [%]	95.1 ± 16.6	94.7 ± 16.2	p = 0.77
PTT [s]	29,8 ± 4,6	29.5 ± 4.6	p = 0.43
Thrombocyte count [10^3/µl]	331.6 ± 130.9	331.0 ± 118.8	p = 0.95
NSCLC	192	196	p = 0.73
SCLC	62	56	p = 0.61
Other malignant disease	27	30	p = 0.68
Other disease	16	14	p = 0.85
Distribution of the biopsy sites			
Trachea	18	16	
Main bronchi	62	57	
Lobe bronchi and intermediate bronchus	156	175	P=0,40
Segmental bronchi	61	48	
Number of exophytic and submucosal lesions in patients with malignancy			
Exophytic	190	183	p= 0.49
Submucosal	91	99	

Patients characteristics are given in absolute and relative frequencies (mean ± SD). The different biopsy sites and the number of exophytic and submucosal tumours are given as absolute values. None of these values differed significantly between the two biopsy groups. PT = prothrombin time, PTT= partial thromboplastin time, NSCLC= non-small cell lung cancer, SCLC = small cell lung cancer

Table 2 **Diagnostic and non-diagnostic biopsies for each biopsy technique in patients with maligancy**

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

Biopsies 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

Table 3 **Procedures used to obtain the final malignant diagnosis after non-diagnostic forceps- and cryobiopsy**

	Forceps (n=42)	Cryoprobe (n=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 punction	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

The procedures used to obtain the final diagnosis after non-diagnostic procedures differed in the forceps- and the cryobiopsy group. There was no preferred second choice method.

Table 4 **Numbers of non-diagnostic biopsies for each biopsy technique**

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

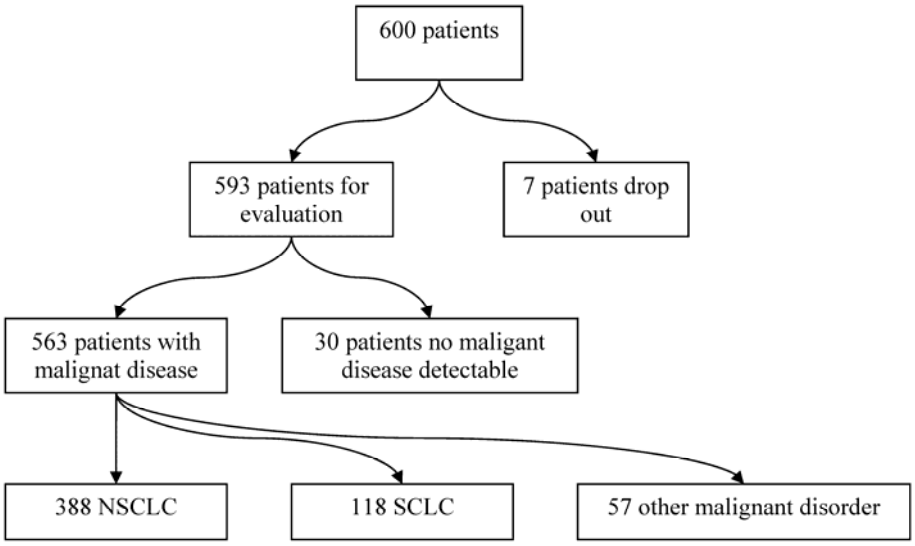
Cryobiopsy was superior over forceps biopsy in the diagnosis of NSCLC and SCLC. CI = Confidence interval.

Table 5 **Biopsy related bleeding for each biopsy group**

Type of bleeding	Forceps	Cryoprobe	P-value
None	91 (30.6%)	59 (19.9%)	p = 0.009
Mild (no intervention)	153 (51.5%)	183 (61.8%))	
Severe (at least one intervention for bleeding control applied)	53 (17.8%)	54 (18.2%)	

Bleeding was more frequent after cryobiopsy than after forceps biopsy.

Figure 1:


600 patients
593 patients for evaluation
7 patients drop out
563 patients with malignat disease
30 patients no maligant disease detectable
388 NSCLC
118 SCLC
57 other malignant disorder