Original Article

Integration of cryobiopsies for interstitial lung disease diagnosis is a valid and safe diagnostic strategy—experiences based on 250 biopsy procedures

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Background: Transbronchial cryobiopsies has become increasingly used in the diagnostic workup in patients suspected of having interstitial lung disease. The procedure is associated with less complications, morbidity and mortality compared to surgical lung biopsies although with a diagnostic yield that is not as high, but close to that of surgical lung biopsies. The aim of the present study was to describe the complications and diagnostic yield and their prognostic factors.

Methods: All patients undergoing transbronchial cryobiopsies at the Department of Respiratory Diseases and Allergy, Aarhus University Hospital, were included in this prospective observational cohort study.

Results: A total of 250 patients were included [61% male, mean age 66 years (range, 22–81 years)]. Pneumothorax was detected in 70 (28%) of the patients, moderate hemorrhage in 53 (21%) and severe hemorrhage in 2 (1%) of the patients. Hemorrhage was associated with central biopsies, but not with anticoagulant therapy. None of the complications were related to lung function, exercise capacity, biopsy or probe size. Only one patient experienced an acute exacerbation. Three-month mortality was 0.4% (1 patient), caused by cancer and unrelated to the procedure. Cryobiopsies contributed to the final diagnosis in 72% of the patients and after multidisciplinary team discussion, a consensus diagnosis was obtained in 82% of the patients. The gender, the total sum of biopsy sizes, number of biopsies and presence of more than 50% alveolar tissue in biopsies increased the diagnostic yield.

Conclusions: Our study confirms that using cryobiopsies in the diagnostic setup for interstitial lung diseases is safe with a limited risk of acute exacerbations and mortality. Cryobiopsies contribute to the diagnosis in the majority of patients.

Keywords: Interstitial lung disease (ILD); cryobiopsy; multidisciplinary team discussions

Submitted Jul 15, 2020. Accepted for publication Dec 30, 2020.
doi: 10.21037/jtd-20-2431
View this article at: http://dx.doi.org/10.21037/jtd-20-2431

Introduction

Interstitial lung disease (ILD) represents a heterogeneous group of diseases characterized by inflammation and/or fibrosis in the lungs and covers more than 200 different disorders. The diagnostics of ILD is multidisciplinary with the integration of clinical, radiological and often pathological findings by ILD specialists. International guidelines recommend a multidisciplinary setting to establish a confident diagnosis (1). Often, invasive procedures are necessary to obtain a confident diagnosis. These procedures include bronchoalveolar lavage (BAL), transbronchial forceps biopsies, cryobiopsies (TBCB) and surgical lung biopsies (SLB). Cryoprobes have been used for many years, initially for debulking of endobronchial lesions. Cryoprobes have proved more effective than forceps biopsies in retrieving sufficient tissue for histological diagnosis without crush artefacts from endobronchial and parenchymal lesions (2-4). Forceps biopsies are less invasive and have fewer complications than SLB and do not require general anaesthesia. However, the diagnostic yield varies from 30% to 75% and is particularly low in ILD (5). SLB has been the gold standard in ILD diagnostics because it allows large tissue samples to be obtained. However, the procedure is associated with a non-negligible morbidity and mortality due to complications, often necessitating prolonged hospitalisation. For the past decade, TBCB has been used in ILD diagnostics and has been shown to have a higher diagnostic yield than forceps biopsies, mainly due to the acquired sample size (6). Thus, TBCB allows a larger volume of alveolar tissue to be obtained without presence of the typical crush artefacts associated with forceps biopsies. Furthermore, complications such as infection, pneumothorax, bleeding and acute exacerbations are significantly fewer in TBCB than in SLB (2,4-8). As a result, TBCB has attracted a growing interest in recent years. However, the recent idiopathic pulmonary fibrosis (IPF) guideline contains no recommendation against or in favour of TBCB in the diagnostic work-up of IPF due to limited evidence (1). Although the recent COLDICE study comparing TBCB with SLB found excellent consistency concerning both histological and clinical diagnosis (9), it is still unclear which factors that increase the diagnostic yield.

The aim of the present study was to present our clinical experiences in using TBCB to obtain diagnostic lung tissue in patients suspected of harboring ILD with specific focus on complications and their risk factors. In this study, we present data from a Danish centre including 250 patients of which data on a cohort of 38 patients have been published earlier (3). We present the study in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/jtd-20-2431).

Methods

All patients undergoing TBCB as part of an investigation for ILD at the Department of Respiratory Diseases and Allergy, Aarhus University Hospital, Aarhus, Denmark, were included in this prospective observational cohort study. Data were collected between November 2015 and August 2019.

Cryobiopsy was performed in general anaesthesia by five experienced interventional pulmonologists using a flexible bronchoscope as previously described in detail (3). The technique behind cryotherapy is the Joule-Thomson effect, where cooling agents (e.g., carbon dioxide or nitric oxide) work under high pressure. When gas expands due to sudden release to atmospheric pressure, the temperature at the probe tip drops quickly and causes the alveolar tissue to freeze and adhere firmly to the tip of the probe, facilitating easy extraction when the cryoprobe is withdrawn (5,7).

The segment for biopsy was selected based on high-resolution computed tomography (HRCT) of the chest at a multidisciplinary team discussion (MDT). Prior to the TBCB, patients were given tranexamic acid intravenously adjusted to body weight to reduce the risk of prolonged bleeding. Patients were continuously monitored with oxygen saturation, blood pressure, electrocardiogram, and exhaled carbon dioxide partial pressure. First, bronchoscopy and BAL were performed. Then a Fogarty balloon was positioned at the entrance of the segmental bronchus leading to an appropriate lung segment for biopsy. The cryoprobes had a diameter of either 1.9 or 2.4 mm. Through the channel of the flexible bronchoscope, the probe was positioned into the selected area using fluoroscopic guidance. The optimal position was achieved approximately 10 mm from the thoracic wall when the probe was facing the wall at a perpendicular angle. The probe was then cooled with CO_2 for five seconds (for the 2.4 mm probe) seven seconds (1.9-mm probe). Then, the bronchoscope and the probe were retracted with the frozen lung tissue attached to the tip of the probe while the Fogarty balloon was inflated. The frozen lung sample was thawed in isotonic saline and fixed in formalin. Four biopsies were obtained when possible. Before and right after the procedure, while the patient was still intubated, lung ultrasonography (LUS) was done to identify

any possible pneumothorax, and fluoroscopy was similarly used right after the procedure to identify pneumothorax. All patients had a sitting or standing chest X-ray done 2 hours after the procedure or sooner if they had symptoms of a possible pneumothorax.

Bleeding complications were categorized as mild if no treatment or treatment with cold saline water and/or Fogarty balloon for less than 2 minutes was required; complication was moderate when cold saline water and/or Fogarty balloon was required for more than 2 minutes or if extra tranexamic acid was used; and severe when patients were hemodynamical unstable and/or required intensive care.

Exclusion criteria to perform TBCB were forced vital capacity (FVC) below 50% of predicted, diffusing capacity for carbon monoxide (DL_{co}) below 35%, body mass index (BMI) above 35, pulmonary hypertension with a tricuspid gradient above 40 mmHg, other cardiac or other comorbidities increasing the risk of complications. Echocardiography was performed in all patients before the procedure.

Classification of the HRCT scans was in accordance with guidelines [American Thoracic Society (ATS)/European Respiratory Society (ERS) IPF guidelines before August 2018 and according to recent ATS/ERS IPF after August 2018] (1,10). Patients who had a definite usual interstitial pneumonia (UIP) pattern on HRCT, reviewed on weekly MDT meetings involving radiologists and pulmonologists, were not considered for the cryobiopsy procedure, neither where patients with a diagnosis of connective tissue disease. If a rheumatologist found no signs of connective tissue disease after investigation upon request, the patient could be considered for the procedure.

The selection of preferred place for biopsy was also determined at this meeting. Possible diagnoses based on HRCT were not collected for this project. TBCB and their morphological pattern were described by an experienced pathologist (LB Madsen) by the same criteria used for SLB as previously described in detail (3). The UIP pattern was categorised as "high confidence" when patchy fibrosis, fibroblastic foci, ± honeycombing were identified. It was categorised as "low confidence" when patchy fibrosis without fibroblast foci (± honeycombing) or fibroblastic foci without associated collagenous fibrosis (± honeycombing) were identified. Only UIP pattern was categorised as high/low confidence. Biopsies were categorised as adequate if 50% or more of the specimen consisted of alveolar tissue.

Both HRCT scans and biopsies were assessed in weekly MDT meetings and were not re-reviewed blinded for this study. The histology diagnosis was based on the pathology report and the discussion of the histology at the MDT meetings. If no specific pattern was seen or if one diagnosis based on histology was not possible due to two or more prominent patterns, the biopsies were classified as "no specific pattern" or "non- contributing". At the MDT meetings, the medical history, symptoms, exposures, pulmonary function tests, 6-minute walk test (6MWT), BAL, HRCT, and histology of each patient was discussed and a final diagnosis was based on this discussion. It was also discussed if the biopsies contributed to the final diagnosis. Cryobiopsies were considered diagnostic if patterns where compatible with the diagnosis after MDT and additional evaluation with either new cryobiopsies or SLB was considered to be unnecessary. If the biopsies were considered normal or with minor, unspecific changes and the changes on HRCT were also minor, new biopsies were not required and the biopsies where not considered diagnostic.

Initially, patients were hospitalised for safety reasons and discharged the following day if no complications occurred. After 10 months, the safety profile allowed an out-patient setting where patients were discharged on the same day of the procedure if no complications occurred.

Baseline demographics, HRCT patterns, size and number of biopsies, histological patterns, their contribution to a confident diagnosis of ILD and potential complications were registered.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethics approval upon request was not necessary as the study was of observational of nature. Individual consent was waived.

Statistics

Data were described and analysed using STATA®16 (StataCorp, College Station, Texas, USA). Data with an approximately normal distribution are presented as mean values ± standard deviation (SD) while data with a non-normal distribution are presented as median values and their interquartile range (IQR) or their total range. Categorical data are presented as proportions of the total population. Continuous data were analysed with the *t*-test or the Mann-Whitney test as appropriate according to their distribution. Categorical data were analysed with the χ^2-test or Fisher's exact test depending on the number of data in each cell. All univariate predictors with a P value <0.20 were

Table 1 Patient demographics and characteristics

Variables	Value
No patients	250
Gender, n [%]	
Females	97 [39]
Males	153 [61]
Age, mean [range]	66 [22–81]
Smoking status, n [%]	
Ex-smokers	126 [50]
Current smokers	42 [17]
Never smokers	81 [32]
Unknown	1 [0.4]
Pulmonary function	
FEV_1, L, mean ± SD	2.37±0.78
FEV_1% predicted, mean ± SD [range]	86±20 [21–133]
FVC, L, mean ± SD	3.13±0.98
FVC% predicted, mean ± SD [range]	91±22 [21–164]
DL_{co}% predicted, mean ± SD [range]	59±14 [35–100]
6MWTD	
Distance, m, mean ± SD [range]	478±117 [100–888]

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; DL_{co}, diffusing capacity of the lungs for carbon monoxide; 6MWTD, six-minute walk test distance; SD, standard deviation.

subsequently entered into a backward multivariate logistic regression model (11).

Results

Demographics

Between November 2015 and August 2019, 250 patients [153 (61%) males] with a suspected diagnosis of ILD underwent bronchoscopy with BAL and TBCB. Mean age was 66 years (range, 22–81 years). The majority of patients were smokers or ex-smokers (67%) (*Table 1*).

Procedure

A Fogarty balloon and fluoroscopic guidance were used in all procedures. In 136 (54%) of the procedures, a cryoprobe size of 2.4 mm was used and in 97 (39%), a cryoprobe size of 1.9 mm. In 3 (1%) patients both cryoprobes were used, first the 2.4 mm and secondly the 1.9 mm due to position difficulties. In the remaining 14 patients (6%) the probe size was not recorded.

Biopsies

In 206 (82%) patients, biopsies were taken from the right lung. Biopsies were taken from the lower lobe in 221 (88%), in the upper lobe in 23 (9%) and/or in the middle lobe/lingula in 14 (6%) patients. In eight patients, biopsies were taken from two different lobes.

The median of the largest diameter of the samples was 8 mm (IQR, 6–9 mm). Pleura was present in 84 (34%) of the cases. TBCB resulted in a specific pattern and contributed to the diagnosis in 180 (72%) of patients. The distribution of histological patterns can be seen in *Table 2*.

Complications

Pneumothorax

Pneumothorax was detected in 70 (28%) patients and 51 (20%) needed a chest tube (*Table 3*). LUS was performed and documented before and after the procedure in 239 (96%) of the patients. Only 19 (27%) of the pneumothorax were detected by LUS immediately after the procedure. In two patients with pneumothorax, one needing a chest tube, LUS was not documented. The remaining 49 cases of pneumothorax (70%) were detected by chest X-ray 2 hours after the procedure. When comparing the two different groups (pneumothorax detected by LUS and chest X-ray), there was no significant difference in the percentage needing a chest tube [LUS: 14 of 19 (74%) patients, chest X-ray: 36 of 49 (73%). Fisher's exact test, P=1].

There were 57 cases of pneumothorax in 221 patients with biopsies taken from a lower lobe (26%), 9 in 23 with biopsies from an upper lobe (39%), and 4 in 14 with middle lobe/lingual biopsies (29%) while there was no pneumothorax in the eight patients where cryobiopsies were taken in two different lobes.

The risk of pneumothorax was lower when biopsies were solely taken from a lower lobe [57 cases of pneumothorax in 213 (27%) patients compared to 13 cases of pneumothorax in 29 (45%) patients in whom biopsies were not taken from one of the lower lobes (P<0.05)].

Of the 70 patients with pneumothorax, cryoprobe size 2.4 mm was used in 35 patients and size 1.9 mm was used in 31 patients. In one patient, both sizes were used and in three patients it was uncertain which size was used. There

Table 2 The histological and clinical diagnosis assigned to patients

Diagnosis	No [%]
(I) Histological diagnosis	
Usual interstitial pneumonia	
High confidence	48 [19]
Low confidence	27 [11]
Hypersensitivity pneumonia	
Fibrotic	30 [12]
Non-fibrotic	6 [2]
Non-specific interstitial pneumonia	
Fibrotic	16 [6]
Cellular	12 [5]
Smoking-related ILD	
Respiratory bronchiolitis-ILD	10 [4]
Desquamative interstitial pneumonia	4 [2]
Organizing pneumonia	10 [4]
Sarcoidosis	6 [2]
Sequela after infection	4 [2]
Eosinophilic pneumonia	3 [1]
Miscellaneous*	15 [6]
No specific pattern or non-contributing	59 [24]
(II) MDT diagnosis	
Idiopathic Pulmonary Fibrosis	
High confidence	46 [18]
Low confidence	27 [11]
Hypersensitivity pneumonia	
Fibrotic	32 [13]
Non-fibrotic	7[3]
Non-specific interstitial pneumonia	
Fibrotic	12 [5]
Cellular	5 [2]
Smoking-related ILD	16 [6]
Cryptogenic organizing pneumonia	7 [3]
Drug-induced ILD	8 [3]
Sarcoidosis	5 [2]
Sequela after infection	6 [2]
Eosinophilic pneumonia	4 [2]
Miscellaneous**	22 [9]
Connective tissue disease-associated ILD	7 [3]
Unclassifiable ILD	46 [18]

*, asbestosis: 2; bronchiolitis obliterans (hematopoietic stem cell transplant): 2; vasculitis: 2; lymphangitis carcinomatosis/lung adenocarcinoma: 2; emphysema: 2; amyloidosis: 1; Langerhans cell histiocytosis X: 1; granulomatous-lymphocytic ILD: 1; chronic hemorrhage: 1; accumulation of lymphocytes in chronic lymphatic leukemia: 1. **, asbestosis: 3; cystic lung diseases: 3; emphysema: 2; bronchiolitis obliterans (hematopoietic stem cell transplant): 2; vasculitis: 2; lymphangitis carcinomatosis/lung adenocarcinoma: 2; amyloidosis: 1; granulomatous-lymphocytic ILD: 1; pleuroparenchymal fibroelastosis: 1; chronic aspergillosis: 1; combined pulmonary fibrosis and emphysema: 1; nodular lymphoid hyperplasia: 1; accumulation of lymphocytes in chronic lymphatic leukemia: 1; interstitial lung abnormality: 1. ILD, interstitial lung disease; MDT, multidisciplinary team discussion.

Table 3 Complications related to cryobiopsies

Complication	Number, n [%]
Pneumothorax	
Total	70 [28]
Need of chest tube	51 [20]
Detected by LUS	19 [27]
Hemorrhage	
Total	138 [55]
Mild	83 [33]
Moderate	53 [21]
Severe	2 [1]
Intensive care	2* [1]
Acute exacerbation	1 [0.4]
3-month mortality	1** [0.4]

*, both patients experienced severe hemorrhage during the procedure, of which one had an acute exacerbation subsequently; **, one patient died due to an underlying cancer disease. LUS, lung ultrasonography.

was no relation between size of cryoprobe and the risk of pneumothorax (P=0.31). There was no relationship between size of biopsy [expressed as the diameter of the largest biopsy among the biopsies taken from the patient being

Table 4 Risk factors for complications and whether the TBCB contributed to the final diagnosis (univariate predictors, P value <0.05 is considered significant)

Predictors (univariate)	Any pneumothorax	Large pneumothorax	Moderate to severe bleeding	Any complication	TBCB contributed to diagnosis
Gender	0.41	0.95	0.14	0.1	0.02
Largest biopsy size	0.91*	0.79*	0.84#	0.64*	–0.08
Largest biopsy >8 mm	0.69	0.81	0.36	0.56	0.02
Sum of biopsy sizes#	0.96	0.57	0.003	0.02	0.23
2.4-mm cryoprobe	0.31	0.68	0.91	0.44	0.82
Left lung biopsy	0.62	0.69	0.35	0.65	0.63
Non-lower lobe biopsy	0.03	0.05	0.15*	0.49	0.57
Upper lobe biopsy	0.21	0.21	0.43*	0.77	0.75
Smoking status	0.11	0.8	0.52	0.65	0.74
Physician	0.08	0.15*	0.06	0.17	0.98
Anticoagulant therapy	–	–	0.77	0.86	0.88
Pleura in biopsy	<0.001	<0.001	<0.001	<0.001	0.92
Age#	0.47	0.25	0.23	0.77	0.77
%FEV_1#	0.47	0.69	0.38	0.79	0.6
%FVC#	0.42	0.56	0.08	0.35	0.33
%DL_{co}#	0.46	0.27	0.17	0.07	0.57
6MWTD#	0.13	0.31	0.39	0.47	0.08

*. Fisher's exact test because of cell(s) with <5 cases; #, Groups compared by Mann-Whitney test. Large pneumothorax = pneumothorax requiring chest tube. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; DL_{co}, diffusing capacity of the lungs for carbon monoxide; 6MWTD, six-minute walk test distance.

larger than 8 mm (the median value of all biopsies taken)] and risk of pneumothorax (P=0.69).

The risk of pneumothorax was not significantly related to lung function (FEV_1, FVC, DL_{co}) in percentage of predicted or 6MWTD (*Table 4*).

Pleura was presented in 51 of the 70 patients with pneumothorax (73%) compared to in only 33 of 180 patients without pneumothorax (18%) (P<0.001). The likelihood of pleura to be present in the biopsies was significantly associated with the physician who performed the procedure (P<0.05); thus, the risk of pneumothorax was also associated with the physician who performed the procedure (P<0.05).

Hemorrhage

In 53 (21%) of the patients, we observed moderate and in 2 (1%) patients, severe bleeding (*Table 3*). The first patient had an acute exacerbation and infection following the severe hemorrhage and was admitted to the intensive care unit (ICU) at our hospital for 16 days before being transferred to the ICU at a local hospital. Prior to the procedure, he was not treated with any anticoagulant drugs. The diagnosis was fibrotic nonspecific interstitial pneumonitis (fNSIP). During hospitalisation he was also diagnosed with small pulmonary and cerebral embolisms (even though he was treated with prophylactic low molecular weight heparin) and chronic lymphocytic leukemia. Hospitalization was prolonged due to rehabilitation.

In the second patient, severe hemorrhage led to cardiac arrest because the lungs were filling with blood and ventilation was difficult. Heart and lung resuscitation was performed successfully and the patient survived without any permanent injuries apart from pain due to broken ribs. The cryoprobe was used to remove the coagulated blood in the lungs. In both these patients, the Fogarty balloon was misplaced when retracting the bronchoscope together with the cryoprobe.

Table 5 Anticoagulant therapy in patients with and without bleeding during the transbronchial cryobiopsies procedure

Anticoagulant therapy	Patients without hemorrhage (N=112), n [%]	Patients with any hemorrhage (N=138), n [%]	Patients with moderate/severe hemorrhage (N=55), n [%]
Acetyl salicylic acid	22 [20]	31 [22]	11 [20]
Thrombocyte inhibitors*	4 [4]	8 [6]	1 [2]
New oral anticoagulants**	5 [4]	7 [5]	3 [5]
Vitamin K antagonists	3 [3]	11 [8]	4 [7]

*, clopidogrel, (prasugrel, ticagrelor); **, apixaban, rivaroxaban.

When pleura was present in the biopsies, the risk of moderate or severe bleeding was lower [only five cases of moderate or severe bleeding in 84 patients with pleura in biopsies (6%) compared to 50 in 166 without pleura in biopsies (30%) (P<0.001)]. Likewise, pneumothorax was significantly associated with a lower risk of moderate or severe bleeding (P<0.001). As a result, the physician with the highest rate of pneumothorax also tended to have the lowest rate of moderate or severe bleeding (15% against 26% for the other physicians as a whole, P=0.056).

The risk of moderate or severe bleeding was not significantly related to lung function [%FEV_1 (P=0.38), %FVC (P=0.08), %DL_{co} (P=0.17)], 6-minute walk test distance (6MWTD) (P=0.39), probe size (P=0.91) or with lower lobe (P=0.34) or non-lower-lobe localisation of the biopsy (P=0.15). Larger biopsies did not increase the risk of moderate or severe bleeding (*Table 4*). In fact, cases of moderate to severe bleeding did on average have smaller biopsies [median sum of biopsy sizes 24 mm (IQR, 20–27 mm) for cases with no or only minor bleeding versus 21 mm (IQR, 17–21 mm) for cases of moderate to severe bleeding (P=0.003)].

Hemorrhage and anticoagulant treatment

In the patients with moderate or severe bleeding, 19 (35%) were on anticoagulant treatment before the bronchoscopic procedure, whereas 34 (30%) of those without bleeding were treated with anticoagulants (P=0.59) (*Table 5*). All patients ceased individual anticoagulant treatment before the procedure according to national guidelines (12). Among patients without any anticoagulant therapy, 50 (30%) had mild bleeding, 34 (21%) had moderate and 2 (1%) patients had severe bleeding. The risk of moderate or severe bleeding was not increased for the patients who had been in anticoagulant or antithrombotic therapy prior to the cryobiopsy procedure [36 cases of moderate to severe bleeding among 164 patients not in anticoagulant or antithrombotic therapy (22%) versus 19 among 86 in anticoagulant or antithrombotic therapy (22%) (P=0.98)].

Acute exacerbation and mortality

Only one patient had an acute exacerbation as described earlier. There were no deaths during the perioperative period or within the first 100 days, apart from the one patient diagnosed with pulmonary cancer and dying from lung cancer on day 88 after the cryobiopsy procedure.

Hospitalisation and reevaluation

On average, patients were hospitalised for 1.5±1.5 days (range, 1–11 days) in the first 10 months (in-patient setting), and 1.1±4.0 days (range, 0–50 days) in the remaining period (out-patient setting). Twenty-five (10%) of the patients were re-evaluated at the hospital within 30 days of which 19 (76%) visits were procedure related (5 with pneumothorax, 5 with pneumonia, 1 with hemoptysis and 8 with chest pain). Of these, 10 were hospitalized. Twenty-eight (11%) of the patients where readmitted within 90 day but none of these were procedure-related (for instance infection, trauma, cholecystitis, non-verified acute coronary syndrome or pulmonary embolism).

Multivariate prediction model for complications

None of the multivariate prediction models were superior to the univariate models for predicting peri-procedural complications and were thus discarded.

Multidisciplinary team discussion

The cryobiopsy sampling contributed to a diagnosis in 180 (72%) of the patients. After the MDT, a consensus

diagnosis was made in 204 (82%) of the patients. The most frequent diagnosis was IPF, hypersensitivity pneumonitis (HP), smoking-related ILD and NSIP (*Table 2*). Of the patients that were not assigned a diagnosis after MDT, eight patients had cryobiopsies performed again (3 where then found to have UIP, 1 fibrotic HP, 1 cryptogenic organizing pneumonia, and 3 unclassifiable). Sixteen underwent SLB (11 with usual interstitial pneumonia, 1 fibrotic HP, 1 respiratory bronchiolitis ILD, 1 asbestosis, 2 unclassifiable); 46 patients had a consensus diagnosis of unclassifiable ILD.

The biopsies contributed more frequently to the diagnosis in females [78 in 97 females (80%) compared to 102 in 153 males (67%), P=0.018]. The larger biopsies [defined as the largest of the biopsies taken from the patient being above 8 mm (the median size for all biopsies taken)] less frequently contributed to the diagnosis [contributed for 55 of 87 with at least one large biopsy (63%) compared to 125 among 163 with all biopsies being below 8 mm (77%), P=0.024]. Slightly more biopsies were taken in patients were TBCB contributed to the diagnosis (P=0.0491) with a mean of 3.8 biopsies (95% CI: 3.4–3.8) compared to 3.6 (95% CI: 3.7–3.9) biopsies. Even though there was a significant association between the presence of pleura in biopsies and at least half of the biopsies having more than 50% alveolar tissue (P=0.04), then the presence of pleura in the biopsies was in itself unrelated to the biopsies leading to a diagnosis (P=0.89). But if at least half of the biopsies were adequate (i.e., more than 50% of alveolar tissue), there was a strong correlation to diagnostic yield (P=0.001). If all biopsies had more than 50% alveolar tissue, as was the case for 109 of the 250 patients, then the biopsies were diagnostic for 85% of the patients with a variation from 81% for the 67 males among the 109 (P=0.02) to 93% for the 42 females (P=0.006).

Multivariate prediction model for diagnostic yield of biopsies

A multivariate prediction model was built by backwards elimination starting from a model including all variables with a P value of 0.20 or less for predicting the diagnostic yield of all the biopsies together. The final model with a P value of 0.0003 consisted of gender, the sum of biopsy sizes, the number of biopsies with more than 50% alveolar tissue but also the number of biopsies with less than 50% alveolar tissue. The model had an area under the receiver operating characteristic (ROC) curve of 0.73. The model predicted that male gender and larger biopsies would reduce the likelihood that the biopsies as a whole would be diagnostic, while a high fraction of biopsies with more than 50% alveolar tissue and a moderate fraction of biopsies with less than 50% alveolar tissue would increase the likelihood where the range of probability would go from a probability of 0.5 for the 10% of cases with the lowest probability to a probability of 0.95 for the 10% with the highest probability.

Discussion

In this prospective cohort of 250 patients undergoing TBCB, we found that the cryobiopsies contributed to the final MDT diagnosis in 72% of all procedures. In 82% of all patients, a consensus diagnosis was obtained after the MDT. The procedure was safe with only one acute exacerbation and one death, not related to the procedure, during 100 days after TBCC.

In the last decade, the use of TBCB has gained ground in the diagnostic workup for ILDs. Topics like complications, risks/benefit ratio, diagnostic yield and diagnostic accuracy have achieved increasing attention and have been discussed frequently (8,13). Some procedural recommendations have been proposed by expert groups (13,14): proper training at a center with experience in the procedure and the management of potential and sometimes severe complications, careful selection of patients, the use of fluoroscopic guidance to find the optimal place to take the biopsy and the use of endobronchial blockers to prevent bleeding. Different centres have a wide variation in preferred procedural techniques, partly due to different set-ups and different workflows and cultures at the centres (2,5). For instance, some centres as ours only use a flexible bronchoscope while others use a rigid bronchoscope. To our knowledge, no randomised studies have compared if the use of either a flexible or rigid bronchoscope affects the complication rate or the diagnostic yield.

Even though data were collected prospectively, missing values were inevitable in a few of the patients (e.g., probe size, 6MWTD). In each of the variables, it counted less than 0.5%. In most cases, only one pathologist revised the biopsies.

Pneumothorax

In our cohort, we had a relatively high number of pneumothorax, partly due to the presence of pleura in 34% of the patients, and partly due to the performance of a routine chest X-ray 2 hours after each procedure.

In some centres, checking for lung sliding with LUS, a supine chest X-ray after the procedure or a chest X-ray if patients develop symptoms of pneumothorax is the only examinations performed to identify pneumothorax (personal communication). Only 19 (27%) of the pneumothorax detected on chest X-ray had in our cohort been detected by LUS right after the procedure, corresponding to a total rate of pneumothorax of 8%. Many of the pneumothorax cases found by the chest X-ray 2 hours after the bronchoscopy did not cause symptoms and would thus not have been diagnosed without routine imaging. Kuse *et al.* routinely performed chest X-ray immediately after the procedure in their cohort of 50 patients and did not detect any pneumothorax (15). Similarly, Sriprasart *et al.* reported a pneumothorax rate of 7% when performing chest X-ray immediately after the procedure (16). Kropski *et al.* and Ussavarungsi *et al.* did not report on how or if they routinely assessed pneumothorax, but their pneumothorax rate was 0% and 1.4%, respectively (17,18). Besides, our higher rate of pneumothorax might also be explained by "air-leak" upon removal of the orotracheal tube which often results in coughing that might increase the intrathoracic pressure and result in a pneumothorax. This could explain why only 27% of the pneumothorax events were detected by LUS, which was performed before extubation.

It is normally attempted to obtain biopsies from the peripheral parts of the lung as this should both reduce the risk of bleeding and increase the likelihood of diagnostic biopsies. In our cohort it was very clear that peripheral biopsies were associated with less risk of moderate to severe bleeding. We also observed that the different bronchoscopists had a different propensity for taking peripheral biopsies. In contrast to others, we did not observe more pneumothorax in patients with biopsies from two different lobes, probably due to the limited number of patients.

Haemorrhage

Severe haemorrhage was rare and mostly mild or moderate and controllable. The severity of haemorrhage is registered differently in different centres (19-23) and the definition of mild, moderate or severe bleeding varies. We chose to define mild haemorrhage as easily controlled bleeding, moderate hemorrhage takes more than 2 minutes to be controlled, and severe haemorrhage results in a hemodynamic unstable patient or requires admission to an ICU. Reassuringly, we have as the first shown that also anticoagulant therapy was not related to the risk of bleeding when proper precautions are taken, probably because guidelines for perioperative management of anticoagulant therapy were meticulously followed and patients with signs of possible pulmonary hypertension were excluded. Notably, both patients with severe bleeding were not treated with anticoagulant therapy. Also, as expected, the risk of haemorrhage increased when biopsies were taken from more central parts of the lungs. Although intuitive, this has never been systematically proven before. One method to avoid severe bleeding might be to use endobronchial radial ultrasonography when performing cryobiopsies in order to minimize the risk of major airway bleeding by visualising and avoiding major blood vessels (24,25).

Our frequency of moderate and severe haemorrhage is similar to previous studies (9,19,20). In the two cases with severe bleeding, the Fogarty balloon had been misplaced when retracting the cryoprobe. This confirms the importance of using endobronchial blockers to prevent life-threatening haemorrhage. In our study, as in other cohorts, bleeding was not related to lung function, exercise capacity, probe size, biopsy size or localization. Contrary to Ravaglia *et al.*, we did not find a relation between bleeding and the place of the biopsy (19).

Acute exacerbations, hospitalisations and mortality

Our results are in accordance with previous studies of TBCB (15-20) and thus confirm that TBCB is a safe procedure as we only observed one acute exacerbation and no procedure-related deaths. For the majority of patients, the procedure can be done as an outpatient procedure with a very low frequency of readmissions.

Diagnostic yield and MDT diagnosis

We experienced a high percentage of 72% of adequate TBCB and a consensus diagnosis in 82% after MDT. This is in accordance with earlier results from our group and with previous studies in which a diagnostic yield between 72% and 87% was found (2,3,13,19). Female gender was related to a higher chance of obtaining a diagnosis; a finding with no obvious explanation. Using logistic regression, we developed a multivariate model to predict the likelihood of diagnostic biopsies better than the individual factors. We found that a combination of gender, sum of the biopsies' size, and number of biopsies could predict the diagnostic yield. We would have expected that adequate biopsies,

no matter their number, had contributed better to the diagnosis. But it appears that the number of biopsies, especially if adequate, and not the size is associated with a higher diagnostic yield. This could be explained by the possibility of seeing different patterns in different biopsies.

The main reason for not obtaining a histologic diagnosis was a biopsy with a non-specific pattern or normal biopsy findings indicating that the biopsy was non-representative. This is further substantiated by obtaining a histological diagnosis upon repeat TBCB or video-assisted thoracoscopic surgery (VATS). Whether more than four biopsies per TBCB procedure can improve the diagnostic yield remains to be shown. We hypothesized that more central or smaller biopsies would explain this finding but surprisingly, we could not in univariable analysis prove that larger or more peripheral biopsies (presence of pleura) contributed more successfully to a diagnosis. However, many of the patients without a histological pattern had less extensive, subtle and very peripheral radiological findings and the biopsies may thus not have been representative.

Risk prediction

Through logistic regression we also attempted to develop a multivariate model to predict the risk of complications better than the individual factors. However, we found no combinations of factors to perform better than the individual factors alone.

Conclusions

Our case series of 250 patients confirms that cryobiopsies contribute to the diagnosis in the majority of patients with suspected ILD and that the procedure for obtaining cryobiopsies is safe with very limited risk of acute exacerbations and mortality. Haemorrhage is related to a central localization of the biopsy site and accordingly, pneumothorax is related to a more peripheral biopsy site and also to non-lower-lobe biopsies. None of these complications were related to lung function, exercise capacity, biopsy or probe size. Gender, the total sum of biopsy sizes, the number of biopsies, and the presence of more than 50% alveolar tissue in at least half of the biopsies increased the diagnostic yield.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at http://dx.doi.org/10.21037/jtd-20-2431

Data Sharing Statement: Available at http://dx.doi.org/10.21037/jtd-20-2431

Peer Review File: Available at http://dx.doi.org/10.21037/jtd-20-2431

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jtd-20-2431). VP, EB and SKW has received grants and personal fees (Erbe, Boehringer and Roche). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethics approval upon request was not necessary as the study was of observational of nature. Individual consent was waived.

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Cite this article as: Kronborg-White S, Sritharan SS, Madsen LB, Folkersen B, Voldby N, Poletti V, Rasmussen TR, Bendstrup E. Integration of cryobiopsies for interstitial lung disease diagnosis is a valid and safe diagnostic strategy—experiences based on 250 biopsy procedures. J Thorac Dis 2021;13(3):1455-1465. doi: 10.21037/jtd-20-2431