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Ultrasound-guided transbronchial cryobiopsy of mediastinal and hilar lesions: a multicenter pragmatic cohort study with real-world evidence

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ABSTRACT

Background Limited data exist on the reliability, efficacy and safety of ultrasound-guided transbronchial cryobiopsy for suspicious mediastinal and hilar lesions. This study shares findings from implementing this method and compares the results with those of the standard endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA).

Methods Patients undergoing diagnostic bronchoscopy for mediastinal or hilar lesions in four Swiss centres were included. The study aims to assess the diagnostic yield and safety of EBUS-guided cryobiopsy compared with EBUS-TBNA. Tunnelling to the target lesion was performed using an electric needle knife (70.8%), a 19 G- (12.4%) or a 22 G needle (16.8%). Cryobiopsies were obtained with a freezing time of 4–7 s (18.2% with a 1.7 mm probe) or 6–10 s (81.8% with a 1.1 mm probe).

Results Altogether, 137 patients were enrolled with a median follow-up of 89 days. The overall diagnostic yield was 56.2% for EBUS-TBNA and 91.2% for cryobiopsies ($p<0.001$). Cryobiopsies increased the diagnostic yield for benign disorders (+28.5%), uncommon tumours (+5.9%) and other metastatic cancer (+0.6%), but not for lung cancer (+0%). For lung cancer (n=27), immunohistochemistry was obtainable in 40.7% of EBUS-TBNA (median of 3 probes [IQR 3 to 3]), significantly lower than cryobiopsy's 88.9% yield (median of 4 probes [IQR 3 to 5]) ($p<0.001$). Adverse events were found in 23.4% of participants; 10.2% had mild to moderate bleeding, 0.7% had pneumonia, and 0.7% (one) of patients had pneumothorax following pneumomediastinum. No deaths or mediastinum infections were observed.

Conclusion Cryobiopsy of mediastinal and hilar lesions improves the diagnostic yield compared with EBUS-TBNA while maintaining a favourable safety profile.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is the standard diagnostic tool for mediastinal and hilar lymphadenopathies; however, recent studies suggest a suboptimal diagnostic yield for benign disorders and uncommon tumours. EBUS-guided cryobiopsy is a promising upcoming tool for mediastinal lesions; however, there is still a lack of data on it.

WHAT THIS STUDY ADDS

⇒ This multicentre pragmatic cohort study showed an overall significantly higher diagnostic yield for mediastinal and hilar EBUS-guided cryobiopsy compared with EBUS-TBNA, especially for benign diseases and uncommon tumours, with a favourable safety profile and high feasibility in patients with and without additional lung parenchymal lesions suspicious of lung cancer.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ EBUS-guided cryobiopsy could be a viable alternative to EBUS-TBNA in specific cases. Further prospective studies with a high sample size are warranted.

INTRODUCTION

Endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration (TBNA) is the current minimal-invasive choice for diagnostic investigation of hilar and mediastinal lymph node staging in lung cancer detected by CT or positron emission tomography (PET).[1 2] However, EBUS-TBNA has demonstrated lower diagnostic yield in uncommon mediastinal tumours and benign diseases,[3 4] as these diagnoses require histopathological rather than cytopathological information.[5 6] Furthermore, despite EBUS-TBNA's high sensitivity for lung cancer, current guidelines[1 2] recommend surgical staging (eg, mediastinoscopy, video-assisted transthoracic surgery (VATS)) if EBUS-TBNA is negative, but high suspicion of mediastinal nodal involvement remains, as some studies have reported clinically relevant false-negative rates for mediastinal staging in non-small cell lung cancer.[7] However, mediastinoscopy is

associated with higher costs[8]; increased risk of adverse events, including airway, oesophageal and nerve injuries, bleeding and longer ventilation time[8 9]; and may not be justifiable in frail patients. Also, nowadays large sample sizes with high tumour cell count and intact cells are required for molecular pathological analysis for targeted therapies for lung cancer, which cannot always be achieved with EBUS-TBNA.[10 11]

Transbronchial cryobiopsy was first described in 2009[12] and used to diagnose interstitial lung diseases and lung cancer.[13 14] Its large sample size of well-preserved lung specimens allows for a similar[15] or even higher[16] diagnostic yield than transbronchial forceps biopsy and has proven to be safe.[15 16] The advancement in imaging for lung cancer has increased the incidence of peripheral lung nodules,[17] for which transbronchial cryobiopsy combined with radial EBUS hold promise as valuable diagnostic tools.[15 18]

Recent studies[3 4] suggest that EBUS-guided cryobiopsy offers a superior diagnostic yield compared with EBUS-TBNA for diagnosing mediastinal lymphadenopathies, especially in benign diseases and rare tumours while maintaining a favourable safety profile. Cryobiopsy of mediastinal and hilar lymph nodes requires transbronchial tunnelling, for which an approach using electric needle knives[3 4] or TBNA needles[19 20] has been described. Due to the lack of data, there is currently no gold standard recommendation.

This pragmatic multicentre cohort study presents data from four Swiss centres that implemented EBUS-guided cryobiopsy. The study aims to assess the diagnostic yield and safety of EBUS-guided cryobiopsy compared with the standard EBUS-TBNA for evaluating mediastinal and hilar lesions of unknown origin in patients with and without lung parenchymal lesions suspicious of lung carcinoma.

MATERIALS AND METHODS

Patient selection and study design

All patients undergoing a diagnostic bronchoscopy for mediastinal and hilar lymphadenopathies with EBUS-guided cryobiopsy and EBUS-TBNA from four study centres during the study period were included in the cohort, patients with additional peripheral pulmonary lung lesions were not excluded. Based on the study design, cryobiopsy was performed at the investigator's discretion but always accompanied by EBUS-TBNA. A CT or PET before bronchoscopy with a mediastinal lesion of ≥1 cm in the short axis was mandatory. Exclusion criteria were (1) age below 18 years or above 90 years, (2) no written informed consent or (3) if TBNA or cryobiopsy were not possible to obtain during bronchoscopy despite efforts. Approval from the Cantonal Ethics Committee of Zurich was obtained (ID 2023–01585). All patients included in this study gave their consent by written general consent.

Patient and public involvement

Patient and public involvement were not applied to this study.

Procedures

Bronchoscopies were performed under moderate to deep propofol procedural sedation with local anaesthetics or general anaesthesia on an outpatient or inpatient basis. A flexible EBUS bronchoscope (190 series; Olympus, Tokyo, Japan) was inserted either through an uncuffed tracheal tube with an inner diameter of 7.5 mm (BronchoFlex, Rüsch, Fellbach, Germany), a laryngeal mask or a rigid bronchoscope (Storz, Tuttlingen, Germany) with an inner diameter between 8.5 mm and 14 mm. After identifying the mediastinal lesion with the EBUS bronchoscope, three TBNA specimens (with 10–12 jabs) were taken with a 19 G, 21 G or 22 G needle, according to the operator's choice, and placed in 0.9% saline for cytopathological assessment. Afterwards, a cryoprobe was placed in the lesion through the insertion canal created by the EBUS-TBNA needle (19G or 22G) or through a small incision created in the tracheobronchial wall with an electric needle knife (papillotomy needle, MTW-Endoskopie, Wesel, Germany) with 40 W and effect 2 or 4 (VIO 200D, APC 2, Erbe, Tübingen, Germany). The EBUS-guided cryoprobe was inserted and activated for 4–7 s for the 1.7 mm probe and 6–10 s for the 1.1 mm probe, respectively. Then, the bronchoscope and cryobiopsy were withdrawn en bloc, thawed in 0.9% saline and fixated in a 4% formalin solution for histomorphological assessment. This process was repeated, based on the investigator's discretion.

Pathologists not involved in the study examined the specimens and measured the long axis of the cryobiopsy samples after fixation. Immunohistochemistry was used to confirm the diagnosis and entity of tumours through marker expression, such as synaptophysin, p40, TTF1 and cytokeratin 7 for lung cancer. Biopsies were considered diagnostic if pathologists could make a definitive diagnosis and non-diagnostic if the lymphatic tissue was without alterations, reactive lymphadenopathy (unspecific) or no lymphatic tissue and no abnormalities were found. The lymphatic tissue with alterations such as granulomas and silico-/anthracosis were defined as benign diseases. Patients with non-diagnostic or benign findings in both TBNA and cryobiopsy received a follow-up with bronchoscopy, mediastinoscopy, VATS, robot-assisted thoracoscopic surgery (RATS), percutaneous needle aspiration, CT, PET or clinical observation including laboratory analysis according to the suspected diagnosis.

After bronchoscopy, pneumothorax was ruled out using lung ultrasound or chest X-ray according to the operator. In rare cases, clinical monitoring was performed instead of an imaging modality. All patients were informed of possible adverse events and encouraged to contact the emergency department if symptoms occurred.

Outcomes

The study aimed to determine the diagnostic yield (primary outcome) and safety profile (secondary outcome) of EBUS-guided cryobiopsy of mediastinal and hilar lesions. Diagnoses were categorised into four groups (lung cancer, benign disorders, other metastatic cancers and uncommon tumours) according to Fan *et al*.[4] Safety was measured by the occurrence of adverse events within 14 days after bronchoscopy such as peri-interventional bleeding, peri-interventional desaturation, tracheal tube dislocation during the cryobiopsy, extensive cough, dyspnoea, haemoptysis, fever, post-bronchoscopy pneumonia, pneumothorax, pneumomediastinum, mediastinitis and death. Bleeding events were evaluated according to the NICE guidelines[21]: no bleeding, mild (requires the suctioning of blood for less than 1 min), moderate (any hemostatic treatment required for example, instillation of vasoactive substances, thrombin or compression of the tunnelling access by the EBUS balloon) and severe (requires red blood cell transfusion, surgical intervention, admission to the intensive care unit or resuscitation). Other outcomes included the dimensions of the harvested tissue with cryobiopsy, histopathological information, transbronchial tunnelling access and the primary conversion rate, which was defined as the ability to obtain tissue from the mediastinal or hilar lesion while excluding non-representative samples without lymphatic tissue or abnormalities.

Statistical analysis

Quantitative data are presented as mean±SD or median (IQR), depending on data distribution. A paired t-test or Wilcoxon signed-rank test was used to account for the dependency of the data for comparisons within patients. Categorical variables are expressed as frequencies (n) and percentages (%). The data were analysed using STATA 18 software (StataCorp LLC, College Station, Texas, USA). The CI was set at 95%, and p values less than 0.05 were considered significant.

RESULTS

Between February 2022 and November 2023, 151 patients with EBUS-TBNA and cryobiopsy of mediastinal or hilar lymphadenopathy were eligible. Thereof, 14 were excluded: 11 did not give consent and tissue extraction by cryoprobe was not possible in five, despite using the electric needle knife (n=4) or the 19G needle (n=1) for tunnelling. In these cases, the force required for cryobiopsy extraction was too high or no tissue was left on the cryoprobe in calcified lymph nodes. Of the five patients, cryobiopsy had to be aborted in three cases, while in the other two cases, the investigators selected another target lymph node region where cryobiopsy extraction was possible. The study flow is shown in figure 1. Overall, 137 patients were included; the baseline characteristics and lesion stations are listed in table 1.

When considering non-representative samples (as displayed in table 2), the primary success rate of EBUS-guided transbronchial cryobiopsy for mediastinal and hilar lesions was 95.0% (defined as representative material from the initial target lesion including those five patients where initial tissue extraction by cryoprobe was not feasible). This is significantly lower than the EBUS-TBNA success rate of 99.3%, representing a difference of 4.3% (95% CI 0.01 to 7.9%, p=0.033). Patients where cryoprobe extraction was not possible were younger than the average cohort (27–57 years old) and were diagnosed with sarcoidosis or an unknown scarring mediastinal mass, which was not further elucidated with mediastinoscopy.

Clinical setting

Mediastinal and hilar lymph lesions were identified with CT in 61 cases (44.5%) and PET in 76 cases (55.5%) a median of 15 days [IQR 7–29] before the investigation. The target lesion was positive for FDG-uptake (SUV>2) in 72 cases (94.7%). 95 patients (69.3%) had an additional peripheral pulmonary lung lesion.

72 patients (52.6%) underwent general anaesthesia, among these, 52 had tracheal tube airway access, 19 laryngeal masks, and one had a rigid bronchoscope. On the other hand, 65 (47.4%) patients underwent moderate to deep sedation, out of which 22 had tracheal tube airway access, 1 had a laryngeal mask, and 42 had no airway access. Six experienced bronchoscopists in four study centres performed the bronchoscopies: examiner 1 (n=70), examiner 2 (n=30), examiner 3 (n=23), examiner 4 (n=9), examiner 5 (n=3) and examiner 6 (n=2).

Cryobiopsy and EBUS-TBNA

Depending on the lymph node size, the 22G TBNA needle was used in 90 cases (65.7%) [median lymph size 3.6 cm^2, IQR 2.6–8.0 cm^2], the 21G needle in 11 cases (8.0%) [median lymph size 3.4 cm^2, IQR 2.0–4.3 cm^2], and the 19G needle in 36 cases (26.3%) [median lymph size 4.7 cm^2, IQR 2.8–11.1 cm^2]. Tunnelling to the target lesion was performed with an electric needle knife in 97 cases (70.8%) [median lymph size 4.6 cm^2, IQR 2.9–8.3 cm^2], with a 19 G needle in 17 cases (12.4%) [median lymph size 3.2 cm^2, IQR 1.9–5.0 cm^2] and with a 22 G needle in 23 cases (16.8%) [median lymph size 2.6 cm^2, IQR 2.2–4.2 cm^2]. Depending on the TBNA needle size used, an electric needle knife was used in 74.4% of patients with a 22G needle, in 100% of patients with a 21G needle, and in 52.8% of patients with a 19G needle. For cryobiopsies, the 1.1 mm cryoprobe was used in 112 cases (81.8%) [median lymph size 3.5 cm^2, IQR 2.4–6.4 cm^2] and the 1.7 mm cryoprobe in 25 cases (18.2%) [median lymph size 6.0 cm^2, IQR 3.5–13.2 cm^2]. Tunnelling with an electric needle knife was used in 66.1% of 1.1 mm and in 92.0% of 1.7 mm cryoprobes. EBUS-TBNA had a median of three aspiration counts [min. 3, IQR 3–3, max. 3], while cryoprobes had a median of four biopsies [min. 1, IQR 3–5, max. 12]. No macroscopic measurements were

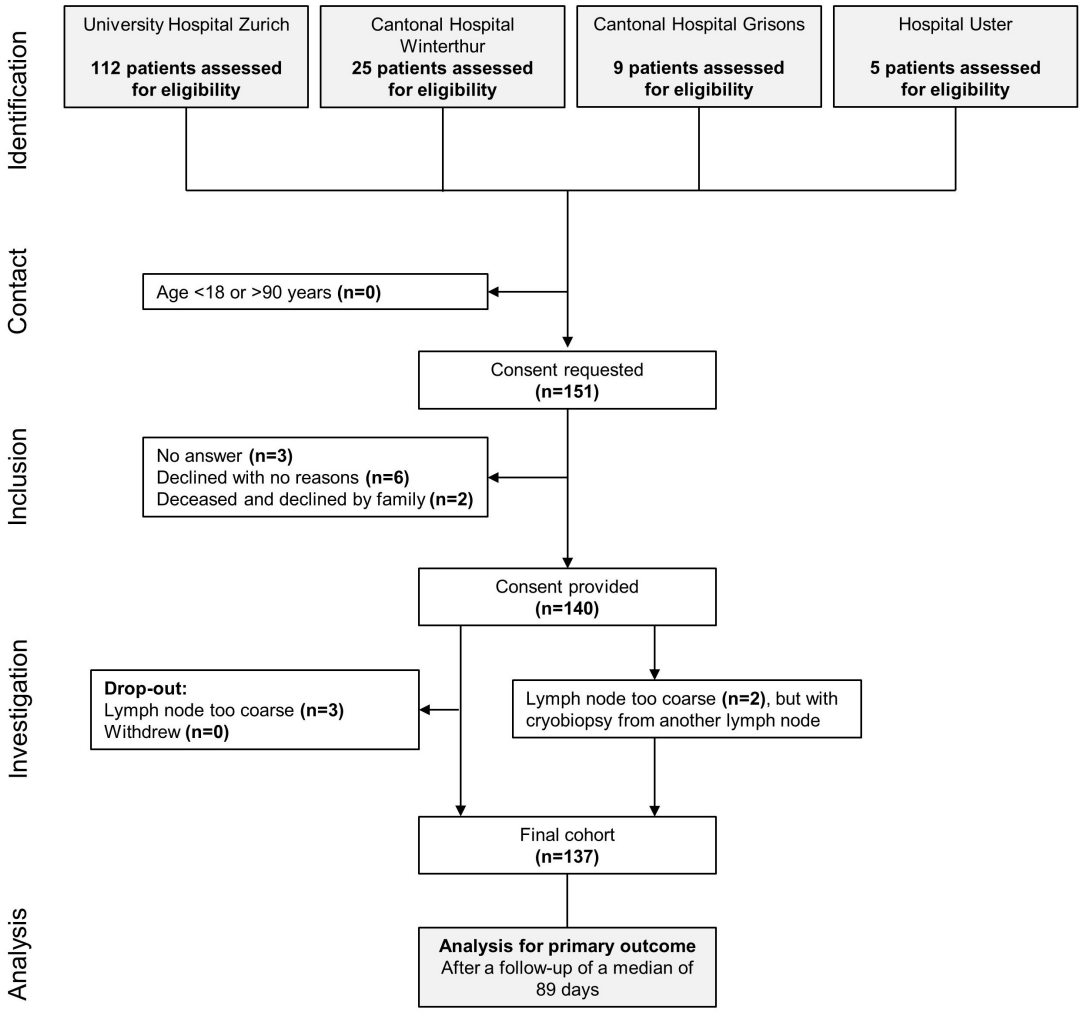

Figure 1 Study flow.

provided for cytologic EBUS-TBNA samples, whereas cryobiopsies were, on average, 5.2±3.0 mm in size (long axis). An illustrative sample size comparison is shown in figure 2.

Follow-up

Follow-up was conducted in 79 patients (57.7%) after a median of 89 days [IQR 21–180]; thereof 7 patients had an inconclusive diagnosis and 72 patients a benign diagnosis in TBNA and cryobiopsy. The methods employed included repeated bronchoscopy (n=4), biopsies from non-pulmonary sites (n=2), surgery (n=9), CT (n=18), PET (n=29) and clinical observation, including laboratory analyses in 34 cases (these methods could overlap in some cases). Clinical observation was only chosen for some cases of sarcoidosis, silico/-anthracosis or reactive lymphadenopathies.

Diagnostic yield

Obtained diagnoses are presented in table 2 and illustrated in figure 3. The overall diagnostic yield was 56.2% for EBUS-TBNA and 91.2% for cryobiopsy (p<0.001). EBUS-TBNA exclusively yielded a definitive diagnosis in one case (0.7%), a lung adenocarcinoma, where tunnelling was performed with the electric needle knife and cryobiopsy showed bronchial and peribronchial tissue without lymphatic tissue, suggesting that the lymph node was missed; however, no adverse events were observed. Cryobiopsy alone provided a definitive diagnosis in 49 cases (35.8%).

The diagnostic yield for cryobiopsiy showed no statistically significant difference across the tunnelling techniques used (94.1% for 19G, 91.3% for 22G and 94.8% for the electric knife; analysis of variance (ANOVA) p=0.646). Similarly, for TBNA no statistically significant difference was observed across the different needle sizes (58.9% for 22G, 45.5% for 21G and 52.8% for 19G; ANOVA p=0.586).

In 81.8%, the 1.1 mm cryoprobe was used, while the 1.7 mm cryoprobe was employed in 28.2%. The specimens obtained with the 1.1 mm cryoprobe were significantly smaller than those retrieved with the 1.7 mm cryoprobe (mean size 4.7 mm [95% CI 4.3 to 5.2] vs 6.8 mm [95% CI 4.9 to 8.8], p=0.001). However, aside from biopsy dimensions, there were no statistically significant differences

Table 1 Baseline characteristics of the cohort

	Summary
N	137
Age, years	59.7±15.8
Sex	
Female	39 (28.5%)
Male	98 (71.5%)
BMI, kg/m^2	26.1±5.1
Weight, kg	78.1±19.2
Height, cm	172±10
Race	
White	131 (95.6%)
Asian	2 (1.5%)
African	3 (2.2%)
Hispanic	1 (0.7%)
Hospital stay	
Outpatient	54 (39.4%)
Inpatient	83 (60.6%)
Smoking status	
Never	53 (38.7%)
Former	55 (40.1%)
Current	29 (21.2%)
Packyears	5 [0 to 30)
Pulmonary Lesion	
Yes	95 (69.3%)
No	42 (30.7%)
Lesion station	
4R	14 (10.2%)
4L	4 (2.9%)
7	83 (60.6%)
10R	4 (2.9%)
10L	0 (0%)
11R	16 (11.7%)
11L	15 (10.9%)
12L	1 (0.7%)
Short axis lymph node, mm	19.8±11.5
Long axis lymph node, mm	29.4±16.0

Percentages are shown as n (%), and numerical values are displayed as mean (SD), or median (IQR).
BMI, body mass index.

between the two cryoprobe sizes in terms of adverse events (20.7% [95% CI 13.2 to 28.2] for 1.1 mm vs 36.0% [95% CI 15.8 to 56.2] for 1.7 mm; p=0.102) or diagnostic yield (90.1% [95% CI 84.6 to 95.8] for 1.1 mm vs 96.0% [95% CI 87.7 to 99.9] for 1.7 mm; p=0.355).

In patients with additional lung parenchymal lesions (n=95), mediastinal EBUS-TBNA showed a lower diagnostic yield (n=59, 62.1%) compared with mediastinal cryobiopsy (n=90, 94.7%, p<0001).

For lung cancer, there was no difference in diagnostic yield (92.6%) between both methods. However, EBUS-TBNA showed a significantly lower diagnostic yield compared with cryobiopsy for benign diseases with 45.5% vs 96.1% (p<0.001) and for uncommon tumours including lymphomas with 47.1% vs 94.1% (p=0.004), respectively.

In cases where lung cancer was detected (n=27), EBUS-TBNA was able to provide suitable material for immunochemistry in 11 cases (40.7%). This was significantly lower than the 24 cases (88.9%) provided by lymph node cryobiopsies (p<0.001).

In 11 cases (8.0%), neither cryobiopsy nor EBUS-TBNA could provide a definitive diagnosis. Five of these patients underwent surgery (two mediastinoscopies, one VATS, one RATS, one thymectomy), four had CT or PET follow-up scans, one had a regular follow-up bronchoscopy and one had a clinical observation. The follow-up showed three cases of sarcoidosis, six reactive lymphadenopathies, one Hodgkin lymphoma, and one lung adenocarcinoma.

Safety profile

Adverse events were observed in 32 patients (23.4%), the most frequent being mild to moderate bleeding in 14 cases (10.2%). The 1.7 mm cryoprobe was used in eight patients with moderate bleeding, the 1.1 mm cryoprobe was used in 2 patients with moderate bleeding and four with mild bleeding. No increased side effects were observed from repeated en-bloc bronchoscope movement through the vocal cords, especially no sore throat, in patients with laryngeal mask airway compared with patients with the endotracheal tube. One patient (0.7%) developed pneumonia after the procedure, while another patient (0.7%) had pneumothorax following pneumomediastinum. The latter patient had a history of asthma and chronic obstructive pulmonary disease (COPD) and needed intermittent high ventilation pressure during bronchoscopy. However, no intervention was required for treatment, and both patients recovered well. No deaths or mediastinal infections were observed during the study. All adverse events are listed in table 3.

DISCUSSION

This multicentre cohort study investigates the diagnostic yield and safety of EBUS-guided cryobiopsy as a novel method for sampling mediastinal and hilar lymph nodes compared with standard EBUS-TBNA. Data from recent studies suggest that the combined approach of cryobiopsy and TBNA compared with TBNA alone could be a better first-line option for patients with undiagnosed mediastinal diseases suspicious for tumours other than lung carcinoma and benign disorders with at least one

Table 2 List of clinical diagnoses

	Final	TBNA	Cryobiopsy	P value
Overall diagnostic yield				<0.001
Definitive diagnosis	–	**77 (56.2%)**	**125 (91.2%)**	
No definitive diagnosis	–	**60 (43.8%)**	**12 (8.8%)**	
Non-diagnostic				<0.001
Lymphatic tissue without alterations	0 (0%)	59 (43.1%)	4 (2.9%)	
Reactive lymphadenopathy (unspecific)	6 (4.4%)	0 (0%)	4 (2.9%)	
No lymphatic tissue, no abnormalities	0 (0%)	1 (0.7%)	4 (2.9%)	
Lung cancer				
Any	27 (19.7%)	25 (18.2%)	25 (18.2%)	1.000
Adenocarcinoma	14 (10.2%)	13 (9.5%)	12 (8.8%)	
Squamous cell carcinoma	7 (5.1%)	7 (5.1%)	7 (5.1%)	
Small cell lung cancer	5 (3.7%)	4 (2.9%)	5 (3.7%)	
Neuroendocrine	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Immunohistochemistry for lung cancer	–	11 (8.0%)	24 (17.5%)	<0.001
Benign disorder				
Any	77 (56.2%)	35 (25.5%)	74 (54.0%)	<0.001
Sarcoidosis	44 (32.1%)	18 (13.1%)	41 (29.9%)	
Silico-/anthracosis	33 (24.1%)	17 (12.4%)	33 (24.1%)	
Other metastatic cancer				
Any	10 (7.3%)	9 (6.7%)	10 (7.3%)	0.319
Clear cell renal cell carcinoma	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Adenocarcinoma (rectum)	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Squamous cell carcinoma (unknown origin)	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Squamous cell carcinoma (pharynx)	1 (0.7%)	0 (0%)	1 (0.7%)	
Melanoma	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Squamous cell carcinoma (skin)	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Adenocarcinoma (prostate)	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Adenocarcinoma (parotid salivary duct)	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Squamous cell carcinoma (oesophagus)	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Thymoma	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Uncommon tumour				
Any	17 (12.4%)	8 (5.8%)	16 (11.7%)	0.004
Lymphoma*	13 (9.5%)	6 (4.4%)	12 (8.8%)	
Sarcomatoid carcinoma (lung)	1 (0.7%)	1 (0.7%)	1 (0.7%)	
Multiple myeloma	1 (0.7%)	0 (0%)	1 (0.7%)	
Sarcoma	1 (0.7%)	0 (0%)	1 (0.7%)	
Atypical carcinoid tumour (lung)	1 (0.7%)	1 (0.7%)	1 (0.7%)	

Final diagnoses were based on follow-up (median of 89 days), cryobiopsy and TBNA samples. Results from cryobiopsy and TBNA are compared with a paired t-test. The final diagnosis assumes that the pathology was present in the investigated lymph node and thus results in a conservative estimate.
*Lymphoma: Hodgkin lymphoma (5x), non-Hodgkin's lymphoma (8x).

mediastinal lesion of 1 cm or longer in the short axis that required diagnostic bronchoscopy. Reasons for the decision to use both methods were undiagnosed mediastinal diseases in patients not highly suspicious for lung cancer or difficult-to-reach solitary pulmonary lesions to prevent re-intervention for further work-up. Furthermore, other reasons were better suitability of the samples for immunohistochemical and molecular analysis.

Cryobiopsy's diagnostic yield was significantly higher (91.2%) than EBUS-TBNA (56.2%), especially in benign

Figure 2 Illustrative macroscopic comparison between three endobronchial ultrasound (EBUS)-guided transbronchial needle aspiration samples (left) and three cryoprobes (right) from the same mediastinal lymph node.

diseases and uncommon tumours, including lymphomas. It is important to note that the diagnostic yield depends on whether reactive lymphadenopathy is defined as a definitive diagnosis, even if confirmed by follow-up. We did not consider reactive lymphadenopathy as a definitive diagnosis; therefore, our diagnostic yield represents a conservative estimate that favours EBUS-TBNA.

A recent prospective study by Zhang *et al*[3] with 197 patients concluded a diagnostic yield of 79.9% for EBUS-TBNA and 91.8% for cryobiopsy. Our yield is slightly lower for EBUS-TBNA but comparable for cryobiopsy. This is probably due to the higher rate of benign diseases and uncommon tumours in our study, compared with Zhang *et al*[3] who reported 24.2% benign diseases, 69.6% common lung cancer and 6.2% uncommon tumours. They further supported our results, showing a significantly lower diagnostic yield with EBUS-TBNA compared with cryobiopsy for benign lesions (53.2% vs 80.9%) and uncommon tumours (25.0% vs 91.7%), with no significant difference for common lung cancer.[3]

Interestingly, we observed an additive increase of 35.8% in diagnostic yield by including cryobiopsy, compared with EBUS-TBNA alone. Maturu *et al*[22] found similar results with an added increase of 43.7% in diagnostic yield by conducting cryobiopsy after inconclusive EBUS-TBNA while showing similarly high number of granulomatous diseases (28.3%) and overall benign diseases (58.7%). However, Maturu *et al*[22] used rapid on-site evaluation, which was not routinely available at our participating centres but could be a valuable tool for stratifying and identifying patients with inconclusive TBNA probes where cryobiopsy may be indicated. In our study, a few patients had a previous bronchoscopy with non-diagnostic EBUS-TBNA, leading to a second bronchoscopy with EBUS-TBNA and cryobiopsy. Consequently, there could be a slight bias that favours cryobiopsy.

No major adverse events were observed. The most common were mild to moderate bleeding, which coincides with the findings of Zhang *et al*,[3] making cryobiopsy a relatively safe method for examining mediastinal and hilar lymphadenopathies. Interventional pulmonologists should be aware of possible bleeding complications associated with these novel techniques. One patient with a history of asthma and COPD required high ventilatory pressure during the procedure, which may have led to pneumomediastinum and subsequent pneumothorax, although no intervention was required.

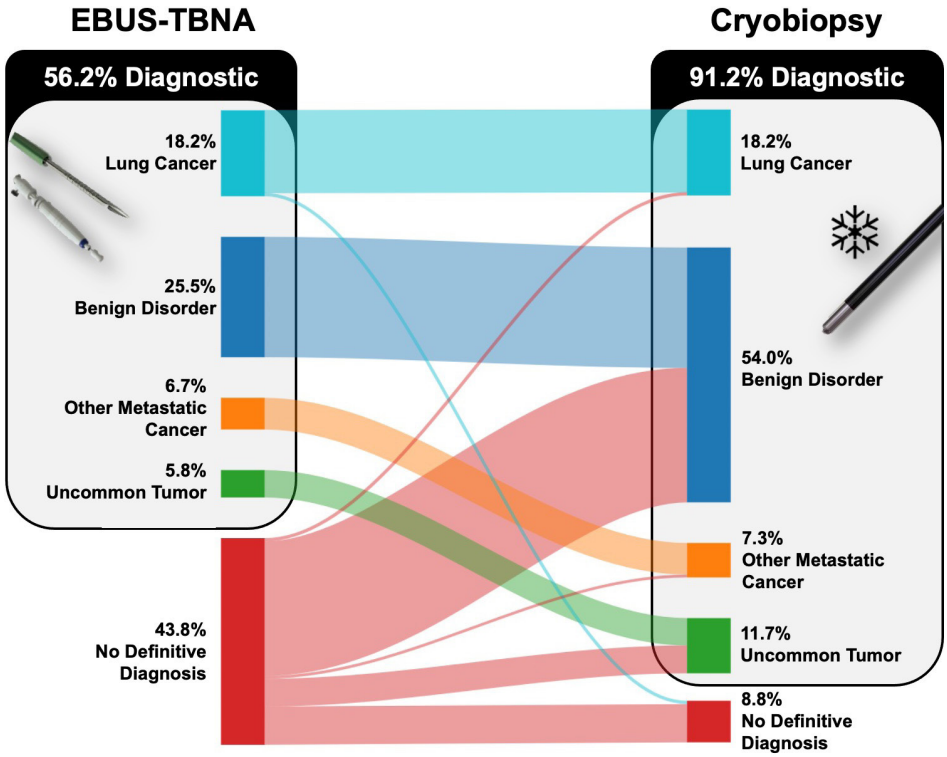

Figure 3 Changes in diagnostic categories with endobronchial ultrasound-guided transbronchial needle aspiration and cryobiopsy. The height of the bars is directly proportional to the relative changes in the whole cohort (n=137). The largest increase in the diagnostic yield of cryobiopsies occurs for benign disorders and uncommon tumours.

Table 3 Adverse events

	Summary
N	137
Overall adverse events	32 (23.4%)
Diagnostics	
Lung ultrasound	103 (75.2%)
Chest X-Ray	44 (32.1%)
Bleeding	
Mild	4 (2.9%)
Moderate	10 (7.3%)
Severe	0 (0%)
Cough	10 (7.3%)
Haemoptysis	6 (4.4%)
Fever	3 (2.2%)
Chest pain	3 (2.2%)
Dyspnoea	2 (1.5%)
Pneumothorax*	1 (0.7%)
Pneumomediastinum*	1 (0.7%)
Airway tube dislocation	1 (0.7%)
Pneumonia	1 (0.7%)
Desaturation	0 (0%)
Mediastinitis	0 (0%)
Death	0 (0%)

Bleeding grade according to the NICE guidelines.[21]
*Pneumothorax and pneumomediastinum occurred in the same patient.

Notably, one case of lung adenocarcinoma was correctly identified by EBUS-TBNA, while cryobiopsy was non-diagnostic. The electric needle knife was used for tunnelling, which might have initially missed the lymph node resulting in no lymphatic tissue being shown in cryobiopsy. As described in studies,[3 19 20] we used an electric needle knife or a 22 G or 19 G TBNA needle for tunnelling according to the lesion size, as there is currently no gold standard. No statistically significant difference was observed for the diagnostic yield of cryobiopsy regarding the different tunnelling methods. However, a disadvantage of the electric needle knife is that it requires additional cauterisation supplies and expertise.[22] We observed five cases where the lymph node was too coarse to obtain a sample with the cryoprobe. Precise tunnelling of the lymph node impacts the diagnostic yield of cryobiopsy, as multiple samples are obtained from the same lesion site. Future studies are warranted to identify the superior tunnelling method and the superior diameter of cryoprobe for mediastinal and hilar lesions. More precise diagnoses could be achieved with tools like cryo-needles[23] or more precise imaging techniques like elastography.

Our analysis reveals that although the 1.7 mm cryoprobe yields larger specimens than the 1.1 mm cryoprobe, this does not correspond to an increased diagnostic yield. Moreover, the reduced flexibility of the 1.7 mm cryoprobe, especially in perpendicular stations such as 4R and 4L, strengthens our recommendation to adopt the 1.1 mm cryoprobe as the primary biopsy tool. In this study, the 1.7 mm cryoprobe was only used in two cases without tunnelling by the electric needle knife (19G n=1, 22G n=1); however, in both cases, the tissue was obtainable and diagnostic. Our study further shows that, compared with EBUS-TBNA (40.7%), cryobiopsy's bigger sample size (5.2±3.0 mm) is more suitable for immunohistochemistry of different tumour markers, such as synaptophysin, p40, TTF1 and cytokeratin 7 (88.9%). Tone *et al*[10] observed that cryobiopsies were more adequate for molecular analysis than EBUS-TBNA when examining lung cancer from the lung tissue. They concluded that EBUS-TBNA provided a smaller sample size with fewer tumour cells and a higher number of damaged cells compared with cryobiopsy.[10] Cryobiopsy could be more appropriate for molecular analysis, as several studies[24 25] have shown that cryobiopsy obtains a large number of DNA and RNA, which is a prerequisite. In our cohort, molecular analysis was not routinely performed.

Another alternative for diagnosing mediastinal lymphadenopathies is intranodal forceps biopsy.[26] A recent meta-analysis[27] showed a higher diagnostic yield using EBUS-guided intranodal forceps biopsy combined with EBUS-TBNA (92%) compared with EBUS-TBNA alone (67%), with an increased diagnostic yield for lymphomas and sarcoidosis and an only slight increase in adverse events compared with EBUS-TBNA alone. Although there is scarce data, a recent retrospective study[28] showed a higher diagnostic yield of EBUS-TBNA combined with cryobiopsy (82.4%) compared with EBUS-TBNA combined with transbronchial forceps (79.4%) and EBUS-TBNA alone (73.5%), for mediastinal lesions. We recommend further prospective studies with large sample sizes to assess the diagnostic yield and safety more accurately between these three methods.

A unique feature of our study is that 69.3% of patients had peripheral lung lesions, with some of them receiving lung parenchymal biopsies during the same examination. This extended indication enables new insights into this patient group, with mediastinal cryobiopsy having a higher diagnostic yield than mediastinal EBUS-TBNA. Interestingly, only 19.7% of patients were diagnosed with lung cancer based on lymph node biopsy, suggesting that EBUS-guided cryobiopsy may provide a 'window into the lung parenchyma' even if the peripheral lung lesions are not directly accessible. This is also useful for lung cancer, as molecular analysis can be reliably generated from lymph node samples obtained with transbronchial cryobiopsy.

Several limitations in this study need to be considered, one being the pragmatic cohort study design with real-world evidence and the low sample size. Given the multicentre clinical setting and the nature of the study design, it was not feasible to blind the pathologists to the results of both EBUS-TBNA and cryobiopsy. Although

the pathologists were not directly involved in the study and those that evaluate TBNA cytology are located on a different floor of the hospital than those who evaluate cryobiopsy histology, we recognise that the lack of blinding represents a limitation of our study design. Considering that only one case was diagnosed using EBUS-TBNA alone (due to the non-representative nature of the cryoprobe sample), we do not believe that the unblinded pathologists were biased towards a higher diagnostic yield for cryobiopsies. Another limitation is that not all follow-ups were confirmed by an invasive procedure with tissue examination and were limited to 89 days post-procedure. Non-invasive follow-ups were done if a benign diagnosis with EBUS-TBNA or cryobiopsy seemed highly likely, and the risks of a more invasive diagnostic outweighed the benefits. If TBNA and cryobiopsy are inconclusive, a follow-up with surgery is recommended. For benign diseases such as sarcoidosis, silico/-anthracosis, reactive lymphadenopathy, sarcoid-like reaction, CT or PET can be used as follow-up. When performing clinical follow-ups, the possibility of misdiagnosis must be considered. As mentioned above, another limitation is that there might be a slight bias in favour of cryobiopsy as some patients received bronchoscopy with EBUS-TBNA and cryobiopsy after a previous non-diagnostic bronchoscopy with EBUS-TBNA; however, this only affected few patients.

This current study is limited by the use of three specimens per lymph node for EBUS-TBNA, whereas a median of four specimens [IQR 3–5] per lymph node was used for cryobiopsy. This difference could favour cryobiopsy's diagnostic yield. Nevertheless, the current CHEST guidelines[29] recommend at least three passes for EBUS-TBNA as a study by Lee *et al*[30] showed no improvement in diagnostic yield after the third EBUS-TBNA pass for suspected lung cancer. A recent retrospective study by Kho *et al*[31] reported that 2–3 cryo-passes with 3.1–4.0 s of freezing time resulted in optimal probe sizes of 4.1–6.0 mm for the 1.1 mm cryoprobe. Earlier studies have described between 1–4 cryo-passes and a freezing time of 3–15 s.[3 4 20 22 31 32] Further prospective studies are needed to evaluate the optimal number of passes and freezing time for mediastinal cryobiopsy.

Three different TBNA needle sizes (19 G, 21 G, 22 G) were used according to the lymph node size, centre preference and possibly personal experience, regardless of the lymph station; however, no statistically significant difference was observed regarding the diagnostic yield across the different needle sizes. A recent systematic review[33] showed no significant difference in the diagnostic yield of the 19 G, 21 G, and 22 G needles in lymph nodes suspected of lung cancer, although the 19 G needle showed higher adequacy for immunohistochemistry and molecular analysis,[33] which could be due to its reported higher tumour cell count.[34] However, a previous systematic review[35] found a significantly higher diagnostic yield for the 19G TBNA needle in sarcoidosis. As there is not yet enough data to determine the superiority of EBUS-TBNA needle size, the current CHEST guidelines[29] and the Joint Indian Chest Society guidelines[36] recommend the operator's choice for the needle size.

It is important to note that the adverse events observed cannot be solely attributed to cryobiopsy. To examine this further, a randomised controlled trial would be needed. However, since no major adverse events were observed, cryobiopsy of mediastinal and hilar lesions appears rather safe even for investigators with low performance numbers, although the overall procedure time might initially be prolonged.

Lastly, in this study, we only examined the most pathological mediastinal or hilar lesions. Examination of additional mediastinal or hilar lymphadenopathies could alter the diagnostic yield of both methods, which could be beneficial if the initial target lesion is too coarse and should be further investigated.

CONCLUSION

A higher diagnostic yield was observed for EBUS-guided transbronchial cryobiopsy of mediastinal and hilar lesions compared with EBUS-TBNA, especially in benign diseases and rare tumours, while maintaining a favourable safety profile and high feasibility, which further supports limited evidence. Prospective, partially blinded studies with larger sample sizes are warranted.

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