Transbronchial mediastinal cryobiopsy in the diagnosis of mediastinal lesions: a randomised trial

Jing Zhang[1], Jie-Ru Guo[1], Zan-Sheng Huang[1], Wan-Lei Fu[2], Xian-Li Wu[1], Na Wu[3], Wolfgang M. Kuebler[4], Felix J.F. Herth[5,6,7] and Ye Fan[1,7]

[1]Dept of Respiratory Disease, Xinqiao Hospital, Third Military Medical University, Chongqing, China. [2]Dept of Pathology, Xinqiao Hospital, Third Military Medical University, Chongqing, China. [3]Dept of Epidemiology, College of Preventive Medicine, Third Military Medical University, Chongqing, China. [4]Institute of Physiology, Charité Universitätsmedizin, Berlin, Germany. [5]Dept of Pneumology and Critical Care Medicine, Thoraxklinik, University of Heidelberg, Heidelberg, Germany. [6]Translational Lung Research Center Heidelberg, University of Heidelberg, Heidelberg, Germany. [7]Felix J.F. Herth and Ye Fan contributed equally to this article as lead authors and supervised the work.

Corresponding author: Ye Fan (fanye2008728@googlemail.com)

Shareable abstract (@ERSpublications)
EBUS-guided transbronchial mediastinal cryobiopsy is a safe and promising novel diagnostic tool for mediastinal diseases that might allow for better histopathological evaluation and advanced testing https://bit.ly/3uiLkiD

Cite this article as: Zhang J, Guo J-R, Huang Z-S, *et al*. Transbronchial mediastinal cryobiopsy in the diagnosis of mediastinal lesions: a randomised trial. *Eur Respir J* 2021; 58: 2100055 [DOI: 10.1183/13993003.00055-2021].

This article has supplementary material available from erj.ersjournals.com

This article has an editorial commentary: https://doi.org/10.1183/13993003.01496-2021

Received: 8 Jan 2021
Accepted: 27 April 2021

Abstract

Background Guidelines recommend endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) as an initial investigatory technique for mediastinal nodal staging in lung cancer. However, EBUS-TBNA can be limited by the inadequacy of intact tissues, which might restrict its diagnostic yield in mediastinal lesions of certain aetiologies. We have previously shown that EBUS-guided transbronchial mediastinal cryobiopsy can provide intact samples with greater volume.

Methods This randomised study determined the diagnostic yield and safety of transbronchial mediastinal cryobiopsy monitored by endosonography for the diagnosis of mediastinal lesions. Patients with a mediastinal lesion of ⩾1 cm in the short axis were recruited. Following identification of the mediastinal lesion by linear EBUS, fine-needle aspiration and cryobiopsy were sequentially performed in a randomised order. Primary end-points were diagnostic yield, defined as the percentage of patients for whom mediastinal biopsy provided a definite diagnosis, and procedure-related adverse events.

Results In total, 197 patients were enrolled and randomly allocated. The overall diagnostic yield was 79.9% and 91.8% for TBNA and transbronchial mediastinal cryobiopsy, respectively (p=0.001). Diagnostic yields were similar for metastatic lymphadenopathy (94.1% *versus* 95.6%, p=0.58), while cryobiopsy was more sensitive than TBNA in uncommon tumours (91.7% *versus* 25.0%, p=0.001) and benign disorders (80.9% *versus* 53.2%, p=0.004). No significant differences in diagnostic yield were detected between "TBNA first" and "Cryobiopsy first" groups. We observed two cases of pneumothorax and one case of pneumomediastinum.

Conclusions Transbronchial cryobiopsy performed under EBUS guidance is a safe and useful approach that offers diagnostic histological samples of mediastinal lesions.

Introduction

Both neoplastic and benign lesions can present as an abnormal mediastinal mass and/or lymphadenopathy. Fast and correct diagnosis of such is mandatory for clinical management and prognosis, and this requires sufficient materials to be obtained for high-quality pathological, genetic, immunological and other assessments [1]. Since the introduction of endobronchial ultrasound (EBUS), enabling real-time supervision of transbronchial needle aspiration (TBNA), this minimally invasive procedure has revolutionised the diagnostic approach for mediastinal diseases, with a shift from more invasive biopsies,

such as mediastinoscopy, to this simple, well-tolerated and cost-efficient process with a far better safety profile [2–4].

The current evidence-based guidelines by the European Respiratory Society, the American Thoracic Society and the American College of Chest Physicians recommend needle-based strategies as the initial approach for the staging of lung cancer [5, 6]. Although EBUS-TBNA provides an excellent diagnostic yield for primary pulmonary malignancies, the limited amount of tissue it retrieves might be insufficient to allow for confident diagnosis of rare tumours or benign mediastinal diseases, which frequently require histopathological rather than cytological samples and an evaluation of the overall background architecture [7, 8]. Hence, despite the considerable success of EBUS-TBNA, there remains significant room for improvement in the diagnosis of mediastinal masses and intrathoracic lymphadenopathies.

Cryoprobes were initially applied within the airways for debulking of endobronchial lesions through freezing and thawing [9, 10]. Because of its ability to harvest a relatively large amount of pulmonary tissue, cryobiopsy has recently been used for the sampling of diffuse lung disease, a setting in which forceps biopsies are of limited value [11, 12]. To improve the acquisition of sufficient high-quality material for histological evaluation, we recently developed a new approach for mediastinal sampling in which we combined transbronchial mediastinal cryobiopsy with the guidance of linear EBUS [13]. This strategy yields more mediastinal tissue for analysis than needle aspiration, with few observed complications. The present study prospectively determined the efficacy and safety of this intervention for sampling of mediastinal material, with diagnostic yield and procedure-related adverse events as the primary outcomes.

Methods

Study design

This study was designed as a randomised trial to evaluate the diagnostic yield and safety of transbronchial mediastinal cryobiopsy in patients with mediastinal diseases (supplementary material). Patients were randomised to receive TBNA followed by cryobiopsy (“TBNA first” group) or cryobiopsy followed by TBNA (“Cryobiopsy first” group) with a distribution of 1:1. Block randomisation was performed using a computer-generated permuted block scheme (blocks of four) by an independent statistician who was not involved in the actual clinical study. Owing to the nature of the intervention, neither participants nor consenting investigators could be blinded to allocation. The study protocol was approved by the ethics committees of the University of Heidelberg and of the Third Military Medical University, and the study design has been registered at the Chinese Clinical Trial Registry (ChiCTR1900025531). All patients provided written informed consent prior to bronchoscopy. The study followed the International Conference on Harmonisation Guideline for Good Clinical Practice and the principles of the Declaration of Helsinki.

Participants

Patients with at least one mediastinal lesion (⩾1 cm in the short axis) and a minimum age of 15 years referred for diagnostic bronchoscopy to the Thoraxklinik Heidelberg or Xinqiao Hospital were eligible for trial entry (supplementary material). Chest computed tomography (CT) or positron emission tomography was performed in all patients prior to bronchoscopy, and the size of the mediastinal lesion was measured. Patients were recruited irrespective of the lesion station. Patients were excluded in cases of mediastinal cysts or abscesses, contraindications to endoscopy or mediastinal biopsy, or potential need for additional procedures other than EBUS (such as endobronchial biopsy). Sample size was calculated based on the largest EBUS-forceps biopsy study so far, which reported an 18% increase in the overall diagnostic yield compared to needle aspiration [14]. Based on these results, a sample size of at least 73 patients per study group was calculated necessary to yield 90% power, assuming a type 1 error of 5%.

Procedures

In all cases, an EBUS procedure was performed by an experienced bronchoscopist under conscious sedation using a convex probe ultrasound bronchoscope (BF-UC260F-OL8 or BF-UC260F, Olympus, Tokyo, Japan) (supplementary material). First, the airways were endoscopically and ultrasonically examined. Once the lesion was detected by EBUS, its location, size and blood supply were recorded. Next, four TBNAs and three cryobiopsies were conducted in random order in all patients as specified below.

EBUS-TBNA was performed as previously described [15]. For transbronchial mediastinal cryobiopsy, a small incision in the tracheobronchial wall adjacent to the mediastinal lesion was made using a high-frequency needle-knife (Olympus KD-31C-1, Olympus). The knife was thereafter replaced by the cryoprobe (Erbe 20402-401, Erbe, Tübingen, Germany), which was introduced into the lesion. The probe was cooled down with liquid carbon dioxide for 7 s, and then retracted with the bronchoscope and the frozen biopsy tissue. Samples were retrieved by thawing in saline and then fixed in formalin.

The pathological specimens were examined by pathologists blinded to the sequence of biopsy techniques. Rapid on-site cytopathological evaluation was not routinely performed. A biopsy was considered diagnostic if the pathological assessment of the sample resulted in a definite diagnosis. Suspicious findings from the biopsy procedures were considered as negative cases. Patients with a plausible benign diagnosis received surgery (mediastinoscopy or surgical lymph node resection) or were followed up with a CT scan after 6 months to exclude a malignant cause. All patients underwent post-procedural chest radiography and were followed for potential EBUS-related adverse events for 4 weeks after biopsy.

Outcomes

The primary end-points of the analysis were 1) the diagnostic yield of transbronchial mediastinal cryobiopsy, defined as the percentage of patients for whom mediastinal biopsy provided a definite diagnosis, and 2) EBUS procedure-related adverse events such as airway bleeding, pneumothorax, pneumomediastinum or mediastinitis. Secondary end-points included sample adequacy and size, and duration of procedure. Samples providing a definite diagnosis or samples with the presence of lymphocytes were considered as adequate, as previously described [16].

Results

Patient characteristics

Between September 2019 and March 2020, 197 patients (117 male, 80 female; 69 from Thoraxklinik Heidelberg, 128 from Xinqiao Hospital) with mediastinal lesions were enrolled in the study and underwent biopsies (figure 1). One patient (from "TBNA first" group) was excluded because he did not tolerate TBNA and the bronchoscopic procedure had to be stopped. Both TBNA and cryobiopsy were successfully conducted in the remaining 196 patients. Baseline data for these patients are presented in table 1. In brief, the mean age of participants was 57.6 years (range 15–88 years) and the average diameter of the lesions was 2.2 cm (range 1.0–8.6 cm). The majority of the lesions were located in the mediastinum: station 7 (n=49), 4R (n=67), 2R (n=4), 4L (n=19) and 2L (n=3); hilar lymphadenectasis were also accessible: station 12R (n=4), 11R (n=14), 10R (n=18), 12L (n=2), 11L (n=7) and 10L (n=10).

Diagnostic yield

The results from the mediastinal biopsies are reported in table 2. All patients completed follow-up except two (one from each group), who declined further investigations (figure 1). Overall, a definite diagnosis

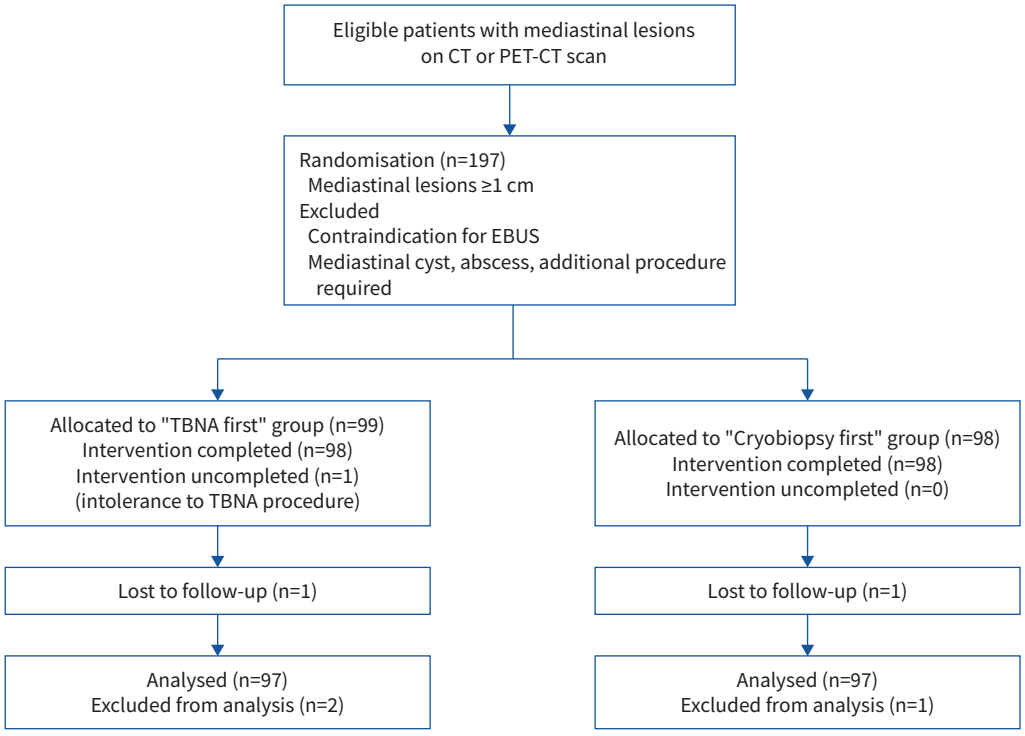

FIGURE 1 Patient flow. CT: computed tomography; PET: positron emission tomography; EBUS: endobronchial ultrasound; TBNA: transbronchial needle aspiration.

TABLE 1 Baseline characteristics

	"TBNA first" group	"Cryobiopsy first" group	p-value
Subjects n	99	98	
Age (years)	57.1±11.4	58.2±13.3	0.53
Sex			0.27
Female	44 (44.4%)	36 (36.7%)	
Male	55 (55.6%)	62 (63.3%)	
Ethnic origin			0.84
Asian	65 (65.7%)	63 (64.3%)	
Caucasian	34 (34.3%)	35 (35.7%)	
BMI (kg·m^{-2})	23.4±2.7	22.6±3.6	0.06
Smoking (pack-year)	18.6±20.6	18.8±21.8	0.95
Lesion size (short axis, cm)	2.3±1.2	2.2±1.1	0.81
Lesion station n			0.61
7	28	21	
12R	2	2	
11R	10	4	
10R	7	11	
4R	33	34	
2R	2	2	
12L	0	2	
11L	2	5	
10L	4	6	
4L	10	9	
2L	1	2	

Data are presented as n (%) or mean±SD, unless otherwise stated. TBNA: transbronchial needle aspiration; BMI: body mass index.

could be established based on the mediastinal specimens in 181 patients (93.3%). Of those, 108 were diagnosed with sarcoidosis and seven with pneumoconiosis. In 152 patients (78.4%), both TBNA and cryobiopsy were able to establish a definite and identical diagnosis. In 26 additional cases in which TBNA biopsy failed to yield a definite diagnosis, the diagnosis could be established from the cryobiopsy (six non-small cell lung cancer (NSCLC), six lymphoma, one seminoma, eight tuberculosis and five sarcoidosis). Conversely, in three NSCLC patients, the diagnosis was only established by TBNA, resulting in an overall diagnostic yield of 79.9% for TBNA and 91.8% for cryobiopsy (p=0.001). Our subgroup analysis demonstrated no significant difference in diagnostic yield between the two techniques for patients with common lung cancer (94.1% for TBNA *versus* 95.6% for cryobiopsy, p=0.58). However, the diagnostic yield of mediastinal cryobiopsy was significantly higher than of TBNA biopsies for uncommon tumours (91.7% *versus* 25.0%, p=0.001) and benign lesions (80.9% *versus* 53.2%, p=0.004).

In four lung cancer patients, more detailed pathological information was available from immunohistochemistry staining of the cryobiopsy samples (revealing two adenocarcinomas with sarcoma-like differentiation, one squamous cell cancer with tuberculosis and one small cell cancer with large cell neuroendocrine carcinoma), which was not possible in the corresponding TBNA samples. In cases of NSCLC, almost all of the cryobiopsy materials (93.3%) were suitable for gene mutation PCR testing, while this ratio was only 73.5% for TBNA, which is consistent with previous reports (p<0.001) [17, 18]. Studies have shown the potentially limited ability of EBUS-TBNA to diagnose and subtype lymphoma [19]. In this study, mediastinal cryobiopsy could establish the diagnosis for the majority of lymphoma patients (87.5%), and all cases diagnosed were successfully sub-classified, which was superior to EBUS-TBNA (supplementary material). Both the overall diagnostic yield and the diagnostic yields of either TBNA or cryobiopsy were similar between the "TBNA first" group and the "Cryobiopsy first" group (table 3). Notably, the likelihood of a cryobiopsy specimen being diagnostic was independent of patient characteristics, lesion size or station (data not shown).

Despite adequate sampling material, 13 patients (6.7%) had a plausible benign yet non-definite diagnosis after both TBNA and cryobiopsy. Three of these cases underwent a cervical mediastinoscopy, one had the lesion surgically resected and one had a regular bronchoscopy. Of these patients, three were diagnosed with NSCLC, one with lymphoma and one with a non-specific benign disorder. The remaining eight patients refused further invasive examination; however, subsequent follow-up CT scans showed either

TABLE 2 Diagnostic yields of TBNA and transbronchial mediastinal cryobiopsy

	Total	TBNA	Cryobiopsy	p-value
Subjects n	194	194	194	
Diagnostic yield n (%)				0.001
No	13 (6.7%)	39 (20.1%)	16 (8.2%)	
Yes	181 (93.3%)	155 (79.9%)	178 (91.8%)	
Common tumour n				
Lung, adenocarcinoma	75	68	72	
Lung, squamous cell	24	24	23	
Lung, large cell	3	3	3	
Lung, NSCLC (NOS)	7	6	5	
Lung, small cell	26	26	26	
Total n (%)	135 (69.6%)	127 (65.5%)	129 (66.5%)	0.58
Uncommon tumour n				
Lung, carcinoid	1	0	1	
Lung, sarcomatoid	1	1	1	
Lymphoma	8	1	7	
Seminoma	1	0	1	
Thymic carcinoma	1	1	1	
Total n (%)	12 (6.2%)	3 (1.5%)	11 (5.7%)	0.001
Benign disorder n				
Sarcoidosis	15	10	15	
Tuberculosis	16	8	16	
Pneumoconiosis	7	7	7	
Total n (%)	47 (24.2%)	25 (12.9%)	38 (19.6%)	0.004

TBNA: transbronchial needle aspiration; NSCLC: non-small cell lung cancer; NOS: not otherwise specified.

regression or stabilisation of lesion size in line with the initially suspected benign diagnosis, and further work-up was not pursued.

Adverse events

No major complications were noted during the procedure, or at the time of the 4-week follow-up (table 4). The most common adverse event observed was minor bleeding, which subsided without the need for intervention. Chest radiography detected two cases (1.0%) of pneumothorax and one case (0.5%) of pneumomediastinum, all of which resolved spontaneously without the requirement for drainage or other interventions.

Sample adequacy and size

Specimens were considered adequate in all TBNA and cryobiopsy samples. The mean diameter and area of the samples retrieved from cryobiopsy were 4.6 mm (range 2.2–8.1 mm) and 10.7 mm^2 (range 3.4–29.8 mm^2), which was even larger than samples obtained from forceps biopsy (supplementary figure S1). No differences in sample size were observed among the different passes of cryobiopsy.

TABLE 3 Diagnostic yields of "TBNA first" procedure and "Cryobiopsy first" procedure

	"TBNA first" group	"Cryobiopsy first" group	p-value
Subjects n	97	97	
Diagnostic yield (overall)			0.77
No	6 (6.2%)	7 (7.2%)	
Yes	91 (93.8%)	90 (92.8%)	
Diagnostic yield (TBNA)			0.37
No	17 (17.5%)	22 (22.7%)	
Yes	80 (82.5%)	75 (77.3%)	
Diagnostic yield (cryobiopsy)			0.6
No	7 (7.2%)	9 (9.3%)	
Yes	90 (92.8%)	88 (90.7%)	

TBNA: transbronchial needle aspiration.

TABLE 4 Procedure-related adverse events

	"TBNA first" group	"Cryobiopsy first" group	p-value
Subjects n	98	98	
Bleeding n			
Grade 2	9	9	
Grade 3	0	2	
Grade 4	0	0	
Total n (%)	83 (84.7%)	86 (87.8%)	0.53
Pneumothorax n (%)	1 (1.0%)	1 (1.0%)	1
Pneumomediastinum n (%)	0 (0%)	1 (1.0%)	1
Death n (%)	0 (0%)	0 (0%)	-

TBNA: transbronchial needle aspiration.

Operation time

The overall procedure time for the EBUS examination was 31.9±9.1 min (range 15.0–53.4 min). The procedural time for the cryobiopsy was slightly longer than for the TBNA (11.7±5.3 min *versus* 9.4±2.6 min, p<0.001).

Discussion

In this prospective comparative dual-centre clinical trial, we tested for the diagnostic yield and safety of transbronchial mediastinal cryobiopsy as a novel sampling technique for diagnostic tissue acquisition from mediastinal lesions and perihilar lymphadenopathies. EBUS-guided transbronchial mediastinal cryobiopsy is safe, and might be superior in the diagnosis of uncommon tumours and benign disorders compared to conventional TBNA. As such, EBUS-guided transbronchial mediastinal cryobiopsy provides a promising and readily implementable strategy for improved diagnosis of mediastinal lesions.

Needle aspiration is currently the standard approach for differential diagnosis of lymph node metastases. Yet, the diagnostic value of TBNA is frequently restricted by the small sample volume available for cytopathological evaluation, which thus provides little information on overall tissue histoarchitecture. This limitation has been proposed to contribute to suboptimal diagnostic yields in rare tumours and benign lesions [20–22]. Furthermore, given the evolving role of targeted therapies and immunotherapy in the treatment of lung cancer, alternative strategies with an increased yield of diagnostic material, allowing for large-scale molecular testing and immunological profiling, may lead to improved diagnosis and earlier introduction of appropriate individualised therapies [23, 24].

Along these lines, previous reports by us and others have demonstrated that the addition of mediastinal forceps biopsy under real-time ultrasound guidance to TBNA significantly improves specimen quality and diagnostic yield, particularly in patients with sarcoidosis and lymphoma [8, 14, 25–27]. EBUS-guided mediastinal forceps biopsy has since been adopted by several interventional pulmonology centres as a routine assessment tool for uncommon neoplasms and benign lesions, despite being potentially beset by the need for specialised skills, prolonged procedure duration and the inability of the forceps to pass the bronchial wall in some cases. However, one of the largest prospective trials of transbronchial forceps biopsy reported a lower diagnostic sensitivity of this technique as compared to needle aspiration in malignant nodes [26].

At present, cryoprobes have been typically used for pulmonary tissue acquisition in interstitial lung diseases, where they have proven superior to forceps biopsy due to the ability to harvest a sufficient sample volume with improved quality for pathological evaluation [11, 12, 28]. In previous work, we have provided evidence that the application of a cryoprobe for transbronchial biopsies in solitary pulmonary lesions is a safe and useful approach to obtain histological samples [28]. In the present study, we demonstrate that transbronchial cryosampling is safe and feasible in mediastinal lesions, and may yield a higher diagnostic sensitivity than the conventional needle aspiration approach. To ensure both acquisition of larger tissue samples and patient safety, we combined transbronchial cryosampling with real-time guidance by EBUS. Similar to the fine-needle, the cryoprobe could be successfully introduced into the mediastinal and hilar lesions in all cases and yield adequate material. Indeed, the size of the material retrieved with the cryoprobe was approximately three times that achieved using mediastinal forceps biopsy.

Importantly, the higher sample volume directly translated into a higher diagnostic yield of the cryoprobe as compared to TBNA. This finding was not attributable to an underperformance of EBUS-TBNA in our cohort, the sensitivity of which was in line with previously reported values [5]. Rapid on-site cytology was not routinely utilised in the present protocol; however, the lack of this has been shown to have no impact on the diagnostic ability of TBNA [29]. As compared to TBNA, and different from the lower sensitivity of forceps biopsy, EBUS-guided transbronchial mediastinal cryobiopsy showed a similar diagnostic yield in lung cancer patients, and, notably, a superior performance in diseases of other aetiologies [26]. Owing to larger specimens, cancer genome sequencing could be performed on cryobiopsy samples in the majority of NSCLC patients, which is better than what could be achieved with TBNA samples. That notwithstanding, it should be noted that in three cases a definite diagnosis of NSCLC was only achieved by TBNA, and not by mediastinal cryobiopsy. While case numbers for this difference may be too small to draw definite conclusions, this finding indicates that a combination of different biopsy tools may further increase diagnostic accuracy of mediastinal lesions. The ability of EBUS-TBNA to accurately diagnose and subtype lymphoma has been questioned because of lesser sampling of core tissue, and recent research has highlighted the value of histological specimens rather than TBNA-acquired cytology for diagnosing lymphoma [30]. In accordance, the large volume of intact tissues from mediastinal cryobiopsy led to improved likelihood ratios for the diagnosis and subtyping of lymphoma in our study, compared to needle aspiration. Furthermore, the diagnostic yield of mediastinal cryobiopsy was not influenced by either the sequence of biopsy approaches or the characteristics of patients and mediastinal lesions, consolidating the robustness and wide applicability of this technique. Interestingly, EBUS-TBNA seemed to have higher diagnostic yield when it was performed before cryobiopsy, which might be due to the reduced specimen quality consequent to cryobiopsy-induced bleeding.

Similar to the previous EBUS report, EBUS-guided transbronchial mediastinal cryobiopsy was generally safe and well tolerated, with only minor adverse events [31]. In theory, relevant complications for this procedure may comprise iatrogenic bleeding, pneumomediastinum, mediastinal infection and pneumothorax. In this study, no signs of severe bleeding, mediastinitis or perioperative death were observed. Pneumomediastinum or pneumothorax as detected by post-procedural radiological examination was evident in 1.5% of participants in the absence of clinical symptoms, and resolved spontaneously without drainage or other interventions. Owing to the study design, it is impossible to attribute these adverse events unambiguously to either needle aspiration or cryobiopsy. Transbronchial mediastinal cryobiopsy was, in any case, associated with a significant prolongation of the bronchoscopic procedure as compared to TBNA. This time difference was primarily attributable to the repetitive retrieval, reinsertion and relocalisation of the EBUS scope for repeated sampling. However, the rather moderate prolongation of the procedure by ~2 min seems acceptable considering the benefits of a higher sample volume acquisition, and suggests that this technique could be managed without greater difficulty by any experienced interventional pulmonologist.

Several limitations need to be considered in the interpretation of the present study. First, three patients with lung cancer were misdiagnosed by transbronchial cryobiopsy, despite the acquisition of more material as compared to TBNA. We assume that this may have been attributable to the fact that cryobiopsies were always conducted at the same location of the lesion, and as such may have missed the diseased tissue. Further studies are needed to evaluate the diagnostic accuracy of cryobiopsies in different regions of mediastinal lesions. Second, only the most suspicious lesions were sampled owing to an initial concern for serious adverse events in case of multinode cryobiopsies. This approach may have limited the diagnostic power of both TBNA and cryobiopsy. However, although systematic surgical mediastinal staging has a better diagnostic yield compared to a site-selective approach in NSCLC, no data are presently available to demonstrate an equal superiority of systematic assessment for EBUS-TBNA [32]. Nevertheless, it should be noted that the accuracy of TBNA might potentially be improved by sampling additional lesions in benign disorders [21]. Finally, the present trial was designed as a crossover study using both fine-needle and cryoprobe in each participant. Because this is the first prospective report on the application of the cryoprobe in mediastinal sampling, it would have been unethical to exclude patients from the standard approach of TBNA. Importantly, the sequence of the two interventions did not affect their diagnostic yield, ruling out systematic effects, while the crossover design allowed for direct comparison of the two techniques in the identical patient cohort.

In summary, the present study reports what is to our knowledge the first use of EBUS-guided transbronchial mediastinal cryobiopsy for the diagnosis of mediastinal lesions. In a dual-centre trial, cryobiopsy proved safe and might offer a higher diagnostic yield as compared to conventional TBNA. Transbronchial mediastinal cryobiopsy might provide an additive value to current diagnostic approaches for mediastinal diseases, specifically in cases of uncommon tumours and benign lesions.

Acknowledgments: We thank the patients, the clinicians, the pathologists and the radiologists for their contribution to the study.

This study is registered as clinical trial ChiCTR1900025531. Because of restrictions in patient consent and institutional review board, raw data collected for this study will regrettably not be made available for the purposes of data sharing.

Author contributions: J. Zhang, J-R. Guo, Z-S. Huang and W-L. Fu contributed equally to this work. J. Zhang and J-R. Guo recruited patients and collected data; Z-S. Huang performed endoscopic procedures; W-L. Fu conducted histological analysis; X-L. Wu helped with data collection; N. Wu performed statistical analysis; W.M. Kuebler analysed the data and drafted the original manuscript; F.J.F. Herth conceived the study, performed endoscopic procedures and drafted the original manuscript; Y. Fan conceived the study, analysed the data and drafted the original manuscript; all authors edited the original manuscript.

Conflict of interest: J. Zhang has nothing to disclose. J-R. Guo has nothing to disclose. Z-S. Huang has nothing to disclose. W-L. Fu has nothing to disclose. N. Wu has nothing to disclose. X-L. Wu has nothing to disclose. W.M. Kuebler has nothing to disclose. F.J.F. Herth reports personal fees for advisory board activities and lecture fees from Pulmonx, Erbe, Olympus and Uptake, outside the submitted work. Y. Fan has nothing to disclose.

Support statement: This work was supported by a grant from the National Natural Science Foundation of China (81873414). Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Wallace MB, Pascual JM, Raimondo M, *et al.* Minimally invasive endoscopic staging of suspected lung cancer. *JAMA* 2008; 299: 540–546.
2 Rintoul RC, Skwarski KM, Murchison JT, *et al.* Endobronchial and endoscopic ultrasound-guided real-time fine-needle aspiration for mediastinal staging. *Eur Respir J* 2005; 25: 416–421.
3 Annema JT, van Meerbeeck JP, Rintoul RC, *et al.* Mediastinoscopy *vs* endosonography for mediastinal nodal staging of lung cancer: a randomized trial. *JAMA* 2010; 304: 2245–2252.
4 Navani N, Nankivell M, Lawrence DR, *et al.* Lung cancer diagnosis and staging with endobronchial ultrasound-guided transbronchial needle aspiration compared with conventional approaches: an open-label, pragmatic, randomised controlled trial. *Lancet Respir Med* 2015; 3: 282–289.
5 American Thoracic Society, European Respiratory Society. Pretreatment evaluation of non-small-cell lung cancer. *Am J Respir Crit Care Med* 1997; 156: 320-332.
6 Detterbeck FC, Jantz MA, Wallace M, *et al.* Invasive mediastinal staging of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). *Chest* 2007; 132: 132: Suppl. 3, 202S–220S.
7 Eapen GA, Shah AM, Lei X, *et al.* Complications, consequences, and practice patterns of endobronchial ultrasound-guided transbronchial needle aspiration: results of the AQuIRE registry. *Chest* 2013; 143: 1044–1053.
8 Franke KJ, Bruckner C, Szyrach M, *et al.* The contribution of endobronchial ultrasound-guided forceps biopsy in the diagnostic workup of unexplained mediastinal and hilar lymphadenopathy. *Lung* 2012; 190: 227–232.
9 Vergnon JM, Huber RM, Moghissi K. Place of cryotherapy, brachytherapy and photodynamic therapy in therapeutic bronchoscopy of lung cancers. *Eur Respir J* 2006; 28: 200–218.
10 Asimakopoulos G, Beeson J, Evans J, *et al.* Cryosurgery for malignant endobronchial tumors: analysis of outcome. *Chest* 2005; 127: 2007–2014.
11 Hetzel J, Wells AU, Costabel U, *et al.* Transbronchial cryobiopsy increases diagnostic confidence in interstitial lung disease: a prospective multicenter trial. *Eur Respir J* 2020; 56: 1901520.
12 Hetzel J, Eberhardt R, Herth FJ, *et al.* Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. *Eur Respir J* 2012; 39: 685–690.
13 Zhang J, Fu WL, Huang ZS, *et al.* Primary mediastinal seminoma achieved by transbronchial mediastinal cryobiopsy. *Respiration* 2020; 99: 426–430.
14 Ray AS, Li C, Murphy TE, *et al.* Improved diagnostic yield and specimen quality with endobronchial ultrasound-guided forceps biopsies: a retrospective analysis. *Ann Thorac Surg* 2020; 109: 894–901.
15 Herth FJ, Annema JT, Eberhardt R, *et al.* Endobronchial ultrasound with transbronchial needle aspiration for restaging the mediastinum in lung cancer. *J Clin Oncol* 2008; 26: 3346–3350.
16 Casal RF, Lazarus DR, Kuhl K, *et al.* Randomized trial of endobronchial ultrasound-guided transbronchial needle aspiration under general anesthesia *versus* moderate sedation. *Am J Respir Crit Care Med* 2015; 191: 796–803.
17 Garcia-Olivé I, Monsó E, Andreo F, *et al.* Endobronchial ultrasound-guided transbronchial needle aspiration for identifying EGFR mutations. *Eur Respir J* 2010; 35: 391–395.

18 Rooper LM, Nikolskaia O, Carter J, *et al.* A single EBUS-TBNA procedure can support a large panel of immunohistochemical stains, specific diagnostic subtyping, and multiple gene analyses in the majority of non-small cell lung cancer cases. *Hum Pathol* 2016; 51: 139–145.

19 Bandyopadhyay D, Panchabhai TS, Mehta AC. EBUS-TBNA for the diagnosis of lymphoma. Still an Achilles heel. *Ann Am Thorac Soc* 2015; 12: 1263–1264.

20 Varela-Lema L, Fernández-Villar A, Ruano-Ravina A. Effectiveness and safety of endobronchial ultrasound-transbronchial needle aspiration: a systematic review. *Eur Respir J* 2009; 33: 1156–1164.

21 Wong M, Yasufuku K, Nakajima T, *et al.* Endobronchial ultrasound: new insight for the diagnosis of sarcoidosis. *Eur Respir J* 2007; 29: 1182–1186.

22 Nakajima T, Yasufuku K, Takahashi R, *et al.* Comparison of 21-gauge and 22-gauge aspiration needle during endobronchial ultrasound-guided transbronchial needle aspiration. *Respirology* 2011; 16: 90–94.

23 Reck M, Rabe KF. Precision diagnosis and treatment for advanced non-small-cell lung cancer. *N Engl J Med* 2017; 377: 849–861.

24 Lantuejoul S, Sound-Tsao M, Cooper WA, *et al.* PD-L1 testing for lung cancer in 2019: perspective from the IASLC Pathology Committee. *J Thorac Oncol* 2020; 15: 499–519.

25 Herth FJ, Schuler H, Gompelmann D, *et al.* Endobronchial ultrasound-guided lymph node biopsy with transbronchial needle forceps: a pilot study. *Eur Respir J* 2012; 39: 373–377.

26 Bramley K, Pisani MA, Murphy TE, *et al.* Endobronchial ultrasound-guided cautery-assisted transbronchial forceps biopsies: safety and sensitivity relative to transbronchial needle aspiration. *Ann Thorac Surg* 2016; 101: 1870–1876.

27 Darwiche K, Freitag L, Nair A, *et al.* Evaluation of a novel endobronchial ultrasound-guided lymph node forceps in enlarged mediastinal lymph nodes. *Respiration* 2013; 86: 229–236.

28 Schuhmann M, Bostanci K, Bugalho A, *et al.* Endobronchial ultrasound-guided cryobiopsies in peripheral pulmonary lesions: a feasibility study. *Eur Respir J* 2014; 43: 233–239.

29 Oki M, Saka H, Kitagawa C, *et al.* Rapid on-site cytologic evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for diagnosing lung cancer: a randomized study. *Respiration*. 2013; 85: 486–492.

30 Plönes T, Mardanzai K, Gafencu D, *et al.* Cytology *versus* histology in the primary diagnosis of lymphoma located in the mediastinum. *Ann Thorac Surg* 2019; 108: 244–248.

31 von Bartheld MB, van Breda A, Annema JT. Complication rate of endosonography (endobronchial and endoscopic ultrasound): a systematic review. *Respiration* 2014; 87: 343–351.

32 Kinsey CM, Arenberg DA. Endobronchial ultrasound-guided transbronchial needle aspiration for non-small cell lung cancer staging. *Am J Respir Crit Care Med* 2014; 189: 640–649.