Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-1005

Reviewer A

Comment 1: Authors needs to add the reference (Abdelghani R, Thakore S, Kaphle U, Lasky JA, Kheir F. Radial Endobronchial Ultrasound-guided Transbronchial Cryobiopsy. J Bronchology Interv Pulmonol. 2019 Oct;26(4):245-249) in their manuscript with a small paragraph discussing/elaborating about use of radial EBUS to locate and select target lung biopsy site before cryobiopsy in patients with ILD which might increase diagnostic yield using the paper as a guide.

Reply 1: We appreciate the reviewer's pertinent advice and encouraging comments. Abdelghani R, et al suggested that the use of radial EBUS to locate and select target lung biopsy site before TBLC might increase diagnostic yield. It was a very meaningful study and provided several inspirations.

Our research differs from this as follows: First, for interstitial lung diseases (ILDs), we may have found some abnormalities and obtain pathological diagnosis in the histopathological examination which R-EBUS did not show any obvious abnormalities in sampling location. Therefore, in our study R-EBUS was mainly used to determine the optimal area for cryobiopsy with the absence of major vessels for ILD cases. For other peripheral pulmonary lesions (PPLs), the lesions detected by R-EBUS were still necessary.

Second, there were several differences of operation method in our study. A flexible thin bronchoscope (BF-P290; Olympus, Tokyo, Japan) was used in our study with 2.0 mm bronchoscope working channel and 4.0mm outside diameter. The R-EBUS probe was inserted through the bronchoscope working channel into the target segment. When the candidate sampling site were detected and confirmed by the R-EBUS probe, the depth of the probe was marked and then the probe was removed. The cryoprobe was guided into the sampling location through the working channel of the bronchoscope and the frozen biopsy program was started.

Third, a total of 137 patients were included in our study which provided more information for research. Not only ILD, we also included PPLs in the study and expanded the application range of transbronchial lung cryobiopsy.

Changes in the text: We added the research conducted by Abdelghani R, et al in the reference and discussed it in the section of discussion (Line 73-75, 220-235; Reference 12).

Comment 2: In the discussion, authors need to discuss about genomic classifier which is currently used to aid in diagnosis of patient with ILD in addition to cryobiopsy and whether such test is currently available in China or not. References to help guide such discussions:

- Kheir F, Uribe Becerra JP, Bissell B, Ghazipura M, Herman D, Hon SM, Hossain T, Khor YH, Knight SL, Kreuter M, Macrea M, Mammen MJ, Martinez FJ, Poletti V,

Troy L, Raghu G, Wilson KC. Use of a Genomic Classifier in Patients with Interstitial Lung Disease: A Systematic Review and Meta-Analysis. Ann Am Thorac Soc. 2022 May;19(5):827-832

-Kheir F, Alkhatib A, Berry GJ, Daroca P, Diethelm L, Rampolla R, Saito S, Smith DL, Weill D, Bateman M, Abdelghani R, Lasky JA. Using Bronchoscopic Lung Cryobiopsy and a Genomic Classifier in the Multidisciplinary Diagnosis of Diffuse Interstitial Lung Diseases. Chest. 2020 Nov;158(5):2015-2025

Reply 2: We appreciate the reviewer's pertinent advice and encouraging comments.

Several studies suggested that a genomic classifier was developed with machine learning and whole transcriptome RNA sequencing using lung tissue for classification of usual interstitial pneumonia (UIP). A systematic review summarized by Kheir F, et al showed that genomic classifier testing predicts histopathologic UIP in patients with ILD with a specificity of 92% and the sensitivity is only 68%. The testing was not widely available. Based on the review, an official ATS/ERS/JRS/ALAT clinical practice guideline for idiopathic pulmonary fibrosis (an Update) and progressive pulmonary fibrosis showed that no recommendation for or against the addition of genomic classifier testing for the purpose of diagnosing UIP in patients with ILD of undetermined type who are undergoing transbronchial forceps biopsy. In China, the genomic classifier testing was not applied for diagnosing ILD routinely.

Changes in the text: A discussion paragraph about the genomic classifier was added and the two papers by Kheir F were also cited in the discussion section of our article (Line236-244; Reference 24).

Comment 3: Authors needs to elaborate more about final ILD diagnosis in their result sections from pathology as well as MDD final diagnosis perspective. What was final diagnois of IPF, sarcoidosis, hypersensitivity pneumonitis,...etc

Reply 3: We appreciate the reviewer's pertinent advice and encouraging comments. We have further summarized and refined the classification of ILD, and merged the original Tables 2 and 3 into a new Table 2 to present the results more clearly.

Changes in the text: The detailed data about final ILD diagnosis of pathology as well as MDD has been presented in renewed Table 2.

Reviewer B

Comment 1: Data presented do not add anything new compared to what is already present in Literature [P.B. Sryma; S. Mittal; N.K. Madan; et al. Efficacy of Radial Endobronchial Ultrasound (R-EBUS) guided transbronchial cryobiopsy for peripheral pulmonary lesions (PPL...s): A systematic review and meta-analysis, Pulmonology, Volume 29, Issue 1, 2023, Pages 50-64, ISSN 2531-0437, doi: 10.1016/j.pulmoe.2020.12.006; Gupta A; Youness H; Dhillon SS; et al. The value of using radial endobronchial ultrasound to guide transbronchial lung cryobiopsy.

J Thorac Dis 2019;11(1):329-334. doi: 10.21037/jtd.2018.10.116].

Reply 1: We appreciate the reviewer's pertinent advice and encouraging comments. Although there have been some published studies on R-EBUS assisted transbronchial lung cryobiopsy, our research has several differences and characteristics. First, for interstitial lung diseases (ILDs), we may have found some abnormalities and obtain pathological diagnosis in the histopathological examination which R-EBUS did not show any obvious abnormalities in sampling location. Therefore, in our study R-EBUS was mainly used to determine the optimal area for cryobiopsy with the absence of major vessels for ILD cases. For other peripheral pulmonary lesions (PPLs), the lesions detected by R-EBUS were still necessary. Second, there were several differences of operation method in our study. A flexible thin bronchoscope (BF-P290; Olympus, Tokyo, Japan) was used in our study with 2.0 mm bronchoscope working channel and 4.0mm outside diameter. The R-EBUS probe was inserted through the bronchoscope working channel into the target segment. When the candidate sampling site were detected and confirmed by the R-EBUS probe, the depth of the probe was marked and then the probe was removed. The cryoprobe was guided into the sampling location through the working channel of the bronchoscope and the frozen biopsy program was started. The procedure was relatively simplified and the radiation machines such as fluoroscopy were not used to avoid patient exposure to radiation. Third, a total of 137 patients were included in our study which provided more information for research. Not only ILD, we also included PPLs in the study and expanded the application range of transbronchial lung cryobiopsy. Further our study was a study summarizing Chinese data, reflecting the development of TBLC technology in China.

Changes in the text: A discussion paragraph about the characteristics of our research was added in the discussion section of our article (Line 220-235)

Comment 2: The objectives and the aim of the study are not clearly specified throughout the manuscript.

Reply 2: We appreciate the reviewer's pertinent advice and encouraging comments. In our study, we aimed to determine the efficacy and safety of TBLC in the diagnosis of peripheral lung diseases in our center. Further, the application value of R-EBUS used to determine the optimal area for cryobiopsy with the absence of major vessels was evaluated in this study. Based on your suggestion, we have added the aim of the study in the part of abstract and introduction in our article.

Changes in the text: Line 27-29, 80-83

Comment 3: It is not clearly specified the number of sampling for each procedure (line 126). Based on ILDs histologic heterogeneity, Literature reported an increased level of TBLC diagnostic accuracy if more than one sampling is made from at least two different sites such as different segments of the same lobe or different lobes [Ruaro, B.; Tavano, S.; Confalonieri; et al. Transbronchial lung

cryobiopsy and pulmonary fibrosis: A never-ending story? Heliyon 2023, 9, e14768. 0.1016/j.heliyon.2023.e14768].

Reply 3: We appreciate the reviewer's pertinent advice and encouraging comments. In our procedure, at least two lung segments and a total of three times cryotherapy biopsies were repeated unless significant bleeding or other serious complications occurred during the operation. The detail has been elaborated and added in our article.

Changes in the text: Line 131-132.