Peer Review File

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Reviewer A

Comment 1: An interesting manuscript in its field, I suggest that the authors add some data regarding the FEV1 of their patients and if they were under oxygen supplement You have to quantify the ratio of LC3II/LC3I. The ratio should be normalized with your internal control.

Reply 1: Thank you for your positive comments on our study. We have added the data regarding FEV1 in both group patients and no difference was identified between them. Furthermore, no patient was under oxygen supplement.
Changes in the Table 1.

Reviewer B

General comment: This was an original article entitled "Transbronchial cryobiopsy using 1.9-mm versus 2.4-mm probe for interstitial lung diseases: analysis from a prospective study".
In this study, cryobiopsy was performed by 1.9mm and 2.4mm cryoprobe under CBCT for patients with ILD. The specimen size, safety and diagnostic efficacy were evaluated. The results showed that 2.4mm probe could provide bigger specimen than 1.9mm probe. However, there were no difference in safety and diagnostic efficacy.
The results were interesting. However, because the endpoints were not described clearly, the interpretation of the results and conclusions was very difficult. Thus, the authors might want to describe the endpoints clearly, especially the primary endpoint. I think that the difference of specimen size (24.6 vs. 22.0 mm2) was not clinically meaningful, and safety and diagnostic efficacy are more important than specimen size. The comments are as below.

Reply: Thank you for your positive comments on our study. And your constructive suggestions greatly improved our manuscript.

Comment 1: What is the primary endpoint in this study? I could not realize the primary endpoint from the manuscript clearly.

Reply 1: The primary endpoints were the incidences of pneumothorax and moderate-severe bleeding, and the second endpoints included diagnostic yields, specimen qualities, cryoprobe re-position rates after CBCT guidance, and procedure duration (please see Page 9, Line 12-15).
Changes in the text: Page 9, Line 12-15.

Comment 2: How was the sample size calculated? I could not realize the sample size calculation

from the manuscript clearly.

Reply 2: The estimated sample size was designed to have approximately 80% power to detect a decrease in pneumothorax rate of 6% (from 9% to 3%), or a decrease in moderate-severe bleeding rate of 10% (from 20% to 10%) after CBCT guidance (please see Page 9, Line 15-18).
Changes in the text: Page 9, Line 15-18.

Comment 3: How many specimens were compared in Table 2. In Table 2, the number of patients was described. However, the number of specimens that were used for statistical analysis was not described.

Reply 3: Thank you for your reminding, the numbers of specimens were 183 and 573 in 1.9-mm and 2.4-mm groups, respectively. We have added this in Table 2.
Changes in the Table 2.

Comment 4: Why were there differences in gross quality and microscopic quality between 1.9mm and 2.4mm probe even though CBCT imaging was used? CBCT imaging was not acquired for repeat cryobiopsies in the same lobe. Should the only specimens obtained immediately after CBCT imaging be used for statistical analysis?

Reply 4: Thank you for this comment. We re-conducted the statistical analysis using the specimens obtained immediately after CBCT to compare gross quality. The result also revealed a significant difference between the two group (unqualified rate: 31.0% (18/58) in the 1.9-mm group vs. 14.0% (26/186) in the 2.4-mm group; P=0.003). In our opinion, the differences in specimen quality between the two sizes of probe may be explained by the cryoprobe nature. In order to yield adequate specimens, bronchoscopists should take some measures to improve the quality of specimens when using a 1.9-mm cryoprobe; these measures may include increasing freezing time, using nitrous oxide as the cryogen (which was shown to be more effective than carbon dioxide), and maintaining the cryogen at a relatively higher pressure level (please see Page 15, Line 15-21).
Changes in the text: none.

Comment 5: Why did pneumothorax occur in only 2.4mm probe? The possible reason of pneumothorax and the characteristics of the patients with pneumothorax should be described.

Reply 5: Thank you for this comment. It is a very important question. The reason of that pneumothorax occurred in only 2.4-mm probe might be the very low risk of pneumothorax and relative small number of patients in the 1.9-mm group. However, the sample size in both groups were enough to demonstrate this issue according to the reported data by previous study (pneumothorax rate: 21.2% when 2.4-mm probe was used, 2.7% when 1.9-mm probe was used; Reference 8). In our opinion, occurrence of pneumothorax depended on the position of biopsy but not probe size. The accurate guidance provided by CBCT significantly decreased the incidence of pneumothorax, even when 2.4-mm probe was used (3.7%). However, in order to

decrease the radiation exposure, CBCT imaging was only performed before the first cryobiopsy, and it was not acquired for repeat cryobiopsies in the same lobe; instead, TBCB was conducted in the same or adjacent segment with the probe advanced at a consistent distance. This might be the possible reason of pneumothorax that occurred in these six patients. We did not found any significant influence factors on pneumothorax by univariate or multivariate analysis.

The characteristics of the patients with pneumothorax have added in the text (please see Page 12, Line 5-16).

Changes in the text: Page 12, Line 5-16.