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Reviewer A

I appreciate the authors for presenting valuable data of 'Diagnostic Efficacy of Cryobiopsy for Peripheral Pulmonary Lesions with Ground-Glass Opacity: A Propensity Score-matched Analysis'. The study investigates the diagnostic efficacy of cryobiopsy compared to conventional biopsy methods for peripheral pulmonary lesions (PPLs) with ground-glass opacity (GGO). Using a propensity score-matched analysis, the study compares the diagnostic performance between patients who underwent cryobiopsy and those who underwent conventional biopsy. The study provides good evidence with reduced bias for the use of cryobiopsy in PPLs with GGOs, as these lesions are difficult to diagnose with existing techniques. Overall, this manuscript has been well written, however, this study can be improved after revision of several concerns.

Major revision

1. Results (paragraph 1) and Supplementary appendix

- In the supplementary appendix, it is mentioned that 270 patients did not undergo cryobiopsy after the introduction of the cryoprobe. The reason for not undergoing cryobiopsy should be clearly stated. Based on these reasons, it would be beneficial to suggest criteria for choosing cryobiopsy in GGO cases.

[Response]

Thank you for your comment. Since this was a retrospective study, the decision to perform cryobiopsy was made by the endoscopist on a case-by-case basis, without predefined criteria.

However, the data showed that the cases which did not undergo cryobiopsy after the introduction of cryobiopsy tended have large lesion, underwent aspiration needle use, were visible on chest radiography, and were classified as "within" using R-EBUS (Supplementary Table S1). These findings suggested the tendency to avoid cryobiopsy in cases that were relatively easy to diagnose via conventional biopsy. In addition to these unbalanced factors, there may be uncertain confounding factors influencing the decision not to perform cryobiopsy. Therefore, we believe that these cases should be excluded from the analysis.

2. Discussion (paragraph 2)

- If bronchial invasion is poor in GGO, it could influence the radial EBUS position relative to the PPLs, leading to a higher proportion of eccentric or adjacent positioning compared to solid lesions with good bronchial invasion. In such PPLs, cryobiopsy demonstrated better diagnostic yield than forceps biopsy. If there is relevant evidence comparing radial EBUS findings between solid lesions and GGO, it would be beneficial to highlight the superiority of cryobiopsy over forceps biopsy in GGO.

[Responce]

Thank you for your insightful review. This propensity score-matched analysis focused on investigating the efficacy of cryobiopsy for PPLs with GGO involving all kinds of R-EBUS detection ("within," "adjacent to," or "invisible"). However, we have included data of subgroup analysis based on R-EBUS findings in Suppelementary Table S2. Based on the subgroup analysis, cryobiopsy seems to be effective for PPLs with GGO, regardless of the R-EBUS findings "within" or "adjacent to" the lesion.

3. Discussion (4th paragraph)

- Suggesting CTR as a risk factor for diagnostic yield could have significant clinical implications. It would be helpful to connect the study findings with other relevant issues, such as indicating that cryobiopsy might have a better diagnostic yield even at low CTR ratios compared to forceps biopsy.

[Responce]

Thank you for your comment. This propensity score-matched analysis focused on investigating the efficacy of cryobiopsy for PPLs with GGO across all ranges of CTR; therefore, it may not be appropriate to determine which CTRs are more effective. However, we have added data of subgroup analysis on CTR in Suppelemtary Table S2 and following text has been added.

P12 L195-197: In the subgroup analysis, the use of cryobiopsy tended to be particularly effective for the lesions ≤20 mm in total size (OR: 5.96 [95% CI: 2.87–12.37]) and nonsolid lesions (OR: 8.54 [95% CI: 3.08–23.7]) (Supplementary Table S2).

4. Discussion (paragraph 6)

- To prevent severe bleeding, the study adopted the two-scope method. However, there are other options to minimize bleeding in GGO, such as reducing the freezing time of cryobiopsy or using different types of cryoprobe (e.g., 1.1 mm cryoprobe). It would be beneficial to suggest these options with relevant evidence, if available.

[Response]

Thank you for your insightful comment. Although reducing freezing time or using thinner (1.1-mm) cryoprobes may potentially contribute to a reduced risk of bleeding, we believe the evidence

is currently insufficient. A previous study showed that freezing time during cryobiospy is associated with specimen size but not significantly associated with bleeding grade (Chen X, et al. *Respiration*. 2022;101:299-306). Additionally, no studies have demonstrated a difference in bleeding risk according to size of cryoprobes. However, controlling bleeding is undoubtedly an important issue in cryobiopsy, and we believe that these points should be clarified in future studies.

Minor

1. Discussion (2nd paragraph)

- Providing numerical data on the diagnostic yield (or HR) between GGO and solid PPLs would be helpful.

[Response]

Thank you for your valuable suggesion. As suggested, we have added the following text as the diagnostic odds ratio between solid PPLs and PPLs with GGO.

P13 L213-215: A previous study reported that the diagnostic OR of solid PPLs to PPLs with GGO was 2.30 (95% CI: 1.07–4.97) in multivariable analysis (12).

2. Discussion (3rd paragraph)

Line 34-35: The diagnostic yield of transbronchial biopsy using forceps combined with R-EBUS and fluoroscopy has been reported to be 47–69% for PPLs with GGO.

- The authors should clearly indicate whether VBN was used in the referenced studies.

[Response]

Thank you for your comment. The text has been revised to refer only to studies combining R-

EBUS, VBN, and fluoroscopy.

P13 L225-226: The diagnostic yield of transbronchial biopsy using forceps combined with R-EBUS, VBN, and fluoroscopy has been reported to be 47–69% for PPLs with GGO (12,32,33).

3. Discussion (paragraph 6)

- Although cryobiopsy shows superior diagnostic yield compared to conventional biopsy in GGO, the risk of bleeding is higher. Providing data (e.g., hazard ratio) comparing the risk of bleeding between cryobiopsy and forceps biopsy with relevant references would be helpful.

[Response]

Thank you for your suggestion. We have already provided data on the difference in the risk of bleeding between conventional biopsy and cryobiopsy in Table 3. We have also discussed the difference in the risk of bleeding between cryobiopsy and forceps biopsy as follows.

P15 L255-259: Regarding adverse events, the incidences of grade 2 and 3 bleeding was significantly higher in the cryo group than in the conventional group (40.5% vs. 8.6% and 2.6% vs. 0.4%, respectively, P <0.001). However, the risk of bleeding in cryobiopsy and conventional biopsy was comparable with the rates reported in our previous study, including all types of PPLs (38.0% vs. 10.2% and 1.5% vs. 0.8%, P <0.001) (20).

4. Discussion (paragraph 6)

- The authors mentioned that GGO has a higher risk of bleeding compared to solid lesions during cryobiopsy. Providing brief data comparing the two (e.g., hazard ratio) would be

beneficial.

[Response]

Thank you for your comment. We have added data on the odds ratio of the risk of bleeding between solid PPLs and PPLs with GGO as follows.

P15 L259-261: In contrast, another study reported that cryobiopsy for PPLs with GGO had a significantly higher risk of bleeding than that of solid PPLs, with an OR of 9.30 (95% CI: 3.40–25.40) noted in multivariable analysis (27).

Reviewer B

Thank you for your interesting paper assessing the diagnostic performance of cryobiopsy in addition to forceps+/-TBNA compared to forceps+/-TBNA in patients with a peripheral nodules containing ground glass undergoing bronchoscopy with R-EBUS and a virtual navigation platform.

My comments are:

1. As you state in your limits section, 'forceps biopsy and/or needle aspiration were performed, followed by cryobiopsy in most cases; thus, the diagnostic results of the cryo group were not based on cryobiopsy alone'. This is a major limitation of your paper and I find it hard to agree on the presented data with the abstract summary statement 'Cryobiopsy improves the diagnostic yield for PPLs with GGO compared with conventional biopsy methods"

Unless you are able to provide specific data regarding the diagnostic yield for the separate components of the procedures (ie forceps/TBNA/cryopbiopsy), which would be very interesting, then it remains unclear which biopsy method in each procedure lead to the diagnosis. The valid conclusion from your data is that the addition of cryobiopsy to forceps +/- needle aspiration appears to improve the diagnostic yield for PPLs with GOO compared to forceps +/- needle aspiration alone.

I find the conclusions stated in the abstract and the discussion misleading. A reader of the abstract alone would draw the conclusion that you are directly comparing cryobiopsy to forceps and this needs to be addressed. The abstract is also missing the key detail of what form of biopsy was undertaken in the conventional group.

[Response]

Thank you for your valuable review. As you noted, it is possible that cryobiopsy specimens did not solely contribute to the diagnosis in all cases, because we did not analyze the specimens separately in this study. Therefore, we have revised the conclusion in the abstract and main text as follows.

P3 L45-46: The combined use of cryobiopsy improves the diagnostic yield for PPLs with GGO compared with conventional biopsy methods.

P16 L292-293: The present study demonstrates that the combined use of cryobiopsy improves the diagnostic yield of PPLs with GGO compared with conventional biopsy methods.

As you suggested, we have also revised the abstract to discuss the use of conventional biopsy method in the cryo group.

P3 L34-37: Patients who underwent only conventional biopsy (forceps and/or needle aspiration) between June 2014 and May 2017 were assigned to the conventional group, whereas those who underwent cryobiopsy with or without conventional biopsy between June 2017 and May 2020 were assigned to the “cryo” group.

2. Your methods section is missing some key information

a. HRCT slice thickness is not stated

[Response]

Thank you for your comment. We have added the information regarding the thickness of HRCT.

P8 L100-101: High-resolution CT (HRCT) with a slice thickness of ≤1.0 mm was performed prior to the procedure in all cases.

b. It is not stated how the (presumably retrospective) review of the HRCT to determine lesion characteristics was performed. Was this performed by an experienced radiologist (or two) blinded to the method of biopsy? Otherwise there is a potential for bias and I would suggest acknowledging this as a limitation.

[Response]

Thank you for your comment. We have stated that in this study, CT findings were evaluated by inexperienced radiologists who were not blinded to the procedure, which could have introduced potential bias.

P16 L275-277: Additionally, CT findings were evaluated by non-radiologists who were not blinded to the procedures, which could have potentially introduced bias.

c. You have not explained the procedural details in the conventional treatment group. Please address this.

[Response]

Thank you for your careful review. We have added the following text to discuss the procedure in the conventional group.

P8-9 L120-121: After R-EBUS probe was withdrawn, conventional biopsy (forceps biopsy and/or needle aspiration) was performed in the conventional group.

3. I am confused about whether you have biopsied the ground glass or solid components of these subsolid lesions

a. Nearly all of your pathology is adenocarcinoma but you haven't provided any breakdown about the presence of in situ, minimally invasive or invasive disease on histopathology. Further detail regarding this would be welcome.

[Response]

Thank you for your suggestion. We have added the surgical pathology findings to the manuscript as follows.

P12 L189-192: Among those with adenocarcinoma as the final diagnosis, surgical resection was performed in 196 cases in the m-cryo group and 179 cases in the conventional group. Among them, adenocarcinoma *in situ* and minimally invasive adenocarcinoma in surgical pathology was diagnosed in 5 and 15 cases in the m-cryo group and 5 and 14 cases in the m-conventional group, respectively.

b. It is not stage how many of the radial EBUS signals were 'conventional' images of the solid components of the PPNs, or the blizzard sign shown in your imaging correlating with the GGO component. Do you have this information?

[Response]

Thank you for your suggestion. We have added the following text regarding the R-EBUS types.

P8 L118-120: The detection of the R-EBUS probe in relation to the lesion was classified as "within," "adjacent to," or "invisible," as defined by Kurimoto et al. (5); the types of R-EBUS signals (blizzard, mixed blizzard, or solid) were also recorded (25).

P11 L183-185: Notably, 79 and 109 cases in the cryo group and 100 and 115 cases in the conventional group showed blizzard signs and mixed blizzard signs on R-EBUS, respectively.

c. Do you have information about the results in patients with pure GGO (were there any)? This would also be pertinent.

[Response]

Thank you for your suggestion. We have added subgroup analysis data for the matched cohort in Supplementary Table S2, specifically addressing the diagnostic performance for nonsolid lesions.

P12 L195-P197 L1: In the subgroup analysis, the use of cryobiopsy tended to be particularly effective for the lesions ≤20 mm in total size (OR: 5.96 [95% CI: 2.87–12.37]) and nonsolid lesions (OR: 8.54 [95% CI: 3.08–23.7]) (Supplementary Table S2).

4. The number of cryobiopsy samples is not stated, nor the size of the cryobiopsy/forceps samples. These two factors may have a significant impact on the yield of the procedure. This information, if available, would be welcome.

[Response]

Thank you for your comment. In this study, cryobiopsy was performed once in most cases, with a maximum of two attempts in some cases. We have added this information to the main text. Unfortunately, we do not have data on specimen size in this study.

P9 L128-129: Cryobiopsy was performed once in most cases, with a maximum of two attempts in some cases.

5. Whilst diagnostic yield has been assessed, the paper would be strengthened by providing data on rates of tissue adequacy for immunohistochemistry and molecular analysis.

[Response]

Thank you for your suggestion. In this study, immunohistochemistry and molecular analysis were not performd in most cases due to the early stage of lung cancer. Therefore, we have decided not to provide the related data in this study. We hope you will agree with our consideration.

Reviewer C

The authors present a propensity score-matched analysis that revealed that cryobiopsy improves the diagnostic yield of PPLs with GGO compared with conventional biopsy

methods. The study is well designed and analyzed. There are some minor points that would benefit from clarification.

Procedure - what type of anesthesia and airway, if any was used? The methods state "All procedures were performed under local anesthesia with moderate to deep sedation" and "All patients in the cryo group were intubated prior to the procedure." Please clarify

[Response]

Thank you for your review. We used a combination of fentanyl or pethidine and midazolam or propofol for sedation, as mentioned in the text (P8 L23-P9 L1). Regarding the airway, we have discussed about the type of intubation tube used as follows.

P9 L122-124: In the cryo group, all patients were intubated with an 8.0-mm inner diameter tracheal tube (Portex Uncuffed Ivory PVC, Oral/Nasal Tracheal Tube, Smiths Medical, Minneapolis, MN, USA) prior to the procedure, and conventional biopsy was performed followed by cryobiopsy in most cases.

Diagnostic yield - was the higher diagnostic yield due only to the use of cryobiopsy or to the additional use of TBNA as "a higher rate of needle aspiration use (22.8% vs. 15.2%, P = 0.028)" was also seen in the m-cryo group? I am infering from the data that it is form the use of cryo, but were there any additional diagnosis gained by the addition of TBNA in the m-cryo group? I would doubt it if the lesion was groundglass, but for those with more solid component/sub-solid, then TBNA may have impacted the results. Please clarify if possible.

[Response]

I apologize for the confusion. A higher rate of TBNA use was seen in the "cryo group" compared

to the conventional group, but this discrepancy was not evident in the "matched cryo (m-cryo) group." The rates of TBNA use between the m-cryo and m-conventional groups were well balanced (18.1% vs. 19.0%, P = 0.905), as shown in Table 1. Therefore, the difference in diagnostic yield between the matched groups should be attributed to the use of cryobiopsy.

We balanced the use of TBNA in the propensity score-matched analysis because a meta-analysis showed that TBNA has a higher diagnostic yield than TBB for PPLs (Mondoni M, et al. *Eur Respir J*. 2016;48:196-204.). Thus, we considered that the use of TBNA can lead to a higher diagnostic yield for PPLs with GGO compared to forceps biopsy.