Peer Review File

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Reviewer A

Interesting porcine study. However, there remain several concerns.

1) It remains unclear, why an animal study has been performed as we have huge data from several clinical studies comparing forceps and cryobiopsy samples. The sample numbers in these studies are even much higher and statistical results seem to be more reliable. The design of the study may be the reason for statistically inconclusive results. Ethical concerns about this animal study may be a consequence of these considerations.

Reply 1: In our experience when conducting and formulating this study, we were unable to generate any studies using the large 2.8mm biopsy forceps alone and in comparison against cryoprobe biopsy.

Changes in the text: We believe no changes in the next are necessitated to address this.

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2) The number of samples is low, this may be the reason why differences are not significant. In Table 4 the differences are striking and may be relevant

Reply 2: Low sample size may explain this. Table 4 demonstrates no statistical difference.
Changes in the text: No changes necessitated.

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3) Crush artifacts are visible in picture 3 but not in picture 1 and 2. This may be highly relevant when samples of ILD subjects are analyzed. The problem of crush artifacts is well described in the literature. What was the definition of crush artifacts for the pathologists?

Reply 3: Crush artifacts are distortion of the tissue architecture and elongation and distortion of the cells. The cell morphology cannot be well evaluated.

Reviewer B

1-The sentence in the abstract and result section is not clear (Out of 32 possible interventions, animal A and B had 18 (56.3%) while animal C had no interventions.
($p<0.001$)). Please clarify

Reply 1: There were multiple outlined interventions. Not all interventions are implemented however many times multiple interventions are used in combination depending on the clinical necessity (eg severity of bleeding). The total permutation of all the interventions that could potentially be done for each patient adds up to 32 combinations.

Changes in the text: Out of 32 potential combinations of interventions, 18 (56.3%) were made.

2-It is important to elaborate on crush artifact when using forceps as compared to cryoprobe. This is the major advantage of using a cryoprobe. Please elaborate more on that

Reply 2: Answer: Crush artifact was only identified in 1 out of 36 slides (#27; CRUL3).

Reviewer C

This manuscript by Abouzgheib et al investigates the utility of TBLB with large forceps to compare with cTBLB.

They showed that the quality of the samples was no significant difference between the three methods. On the other hand, they also showed that there was a significant difference in incidences of bleeding and interventions when comparing transbronchial cryobiopsy and large forceps biopsy.

The manuscript is of interest but there are multiple concerns which preclude publication of the report in its current form.

Some details need to be clarified.

Major revision

1)You described that utilization of cryoprobe biopsy needs to be performed under rigid bronchoscopy and subsequently required operator expertise. However, Inomata et al reported that they performed these procedures without using rigid bronchoscopy and they could have an acceptable safety profile and diagnostic yield in patients.

Reply 1: We agree that there are reported instances where cryoprobe biopsy is performed under standard flexible bronchoscopy. However, most reported literate reports using rigid bronchoscopy under ideal circumstances with a vast minority of cryoprobe biopsies being performed under flexible bronchoscopy. We do note that it can also be completed under a double-lumen endotracheal tube as well.

Changes in the text: For those not candidates for rigid bronchoscopy (e.g. unfavorable anatomy for neck extension) cryobiopsy can be obtained safely when an occlusion balloon can be simultaneously loaded via double-lumen endotracheal tube.

If you have any comments on these differences, please let me know.

https://openres.ersjournals.com/content/erjor/6/2/00008-2020.full.pdf

Reply to linked article above: The linked study demonstrates the already widely utilized practice of endobronchial balloon blockade as pre-emptive management for localization and cessation of airway bleeding. Our study is aimed at demonstrating the differences in yield, bleeding complication, and pathologic quality between a large 2.8mm forceps and a standard cryoprobe. As such, the study is predicated on the known elevated bleeding risk with cryoprobe which is undertaken for the benefit of a better pathologic specimen.

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2) In order to reduce lung collapse after biopsy, we use suction to expand lung tissue. Have you performed such an action?

Reply 2: If you could kindly please rephrase your question we would be greatly appreciate it because as is its unclear how to address. Application of suction endobronchial will induce atelectasis. To our understanding, only application of suction via tube thoracostomy will lead to expanded lung tissue. However, we only place tube thoracostomies in the setting of clinically significant pneumothorax.

Changes in the text: None

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3)You mention in the manuscript the histologic quality of each sample was assessed under a low power field. And then, as you mention that this assessment used a score which accounted for, by order of importance, the percentage of alveolated tissue, percentage of bronchial tissue, specimen size, presence of crush artifact, and presence of hemorrhage. Please explain how to evaluate each condition about these categories more in detail.

Awaiting comment from our pathology division

Reply 3: **Specimen**

All biopsy samples were placed and fixed in formalin, which were processed in paraffin blocks and stained with hematoxylin and eosin in an outside facility in order to eliminate bias and maintain blindness of both pathologists. Each sample was labeled with A, B, or C letter depending on which animal, followed by targeted lobe and then by number of sample. The slides were then provided to two pathologists separately who were blinded to the corresponding biopsy tool. If the sample contained multiple fragments, the number of fragments was recorded and then clumped together in a ~~cell~~ block.

The histologic quality of each sample was assessed using a score that accounted, by order of importance, for area of specimen size, alveolated tissue, size of bronchial tissue, presence of crush artifact and presence of hemorrhage. The large samples with high alveolated tissue/bronchial tissue ratio, and minimal crush artifact and hemorrhage were given a high score. The small sample with low alveolated/bronchial tissue ratio with significant crush artifact and hemorrhage were given a low score. (Table 2)

Overall quality	Description	Alveolar tissue area	Bronchial tissue	Crush artifact	Hemorrhage
4	Good	>= 4 mm^2	No or scant	No	No or Scant
3	Acceptable	>= 2 mm^2	No or scant	No or scant	Focal
2	Scant alveolar tissue	< 2mm^2	Present	Focal	Focal
1	No alveolar tissue	0	Present	NA	Present

4)The quality of the pathology depends on whether it is interstitial pneumonia, lung cancer, or some other lesion.

Since the quality of the tissue specimen varies depending on the purpose, we cannot say that forceps are immediately better based on this study alone. Shouldn't that point be mentioned in the discussion or limitation?

Reply 4: We agree that tissue specimen varies depending on the pathologic indication and as such, certain types of tissue have different diagnostic yields, even with similar diagnostic techniques. However, our study was not aimed at pathologic diagnosis. We wanted to evaluate specimen quality. The study was not aimed at pathologic diagnosis as the animal models we used were presumably healthy.

Changes in the text: Note that this study was not powered at attaining pathologic diagnosis, but rather to characterize specimen quality.

Minor revision

1)The quality of the language needs to be improved. There are some misspelled and incorrect words. Please request an English editing service to make corrections again.

Reply 1: We made edits according to feedback from English editors.
Changes in the text: Various, across all text

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2)There are too many figures and tables.

How about combining a few more or moving some of the data to supplements?

Reply 2: We think it is important for readers to have all data available, but happy to consolidate if required by editors

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Further Comment from Reviewer C

I think the manuscript has been mostly revised. However, I would like to add a few comments.

1) You described that utilization of cryoprobe biopsy needs to be performed under rigid bronchoscopy and subsequently required operator expertise. However, Inomata et al reported that they performed these procedures without using rigid bronchoscopy and they could have an acceptable safety profile and diagnostic yield in patients.

Reply 1: We agree that there are reported instances where cryoprobe biopsy is performed under standard flexible bronchoscopy. However, most reported literate reports using rigid bronchoscopy under ideal circumstances with a vast minority of cryoprobe biopsies being performed under flexible bronchoscopy. We do note that it can also be completed under a double-lumen endotracheal tube as well.

ADDITIONAL COMMENT: The authors seem to be misunderstanding the situation, so I dare to make a comment. The reference article presented previously does not refer to balloon occlusion using a double lumen tube. Please check the contents again and correct them appropriately.
Reply : addressed and added as reference in conclusion paragraph line 263- we appreciate the suggestion

3) You mention in the manuscript the histologic quality of each sample was assessed under a low power field. And then, as you mention that this assessment used a score which accounted for, by order of importance, the percentage of alveolated tissue, percentage of bronchial

tissue, specimen size, presence of crush artifact, and presence of hemorrhage. Please explain how to evaluate each condition about these categories more in detail.

Reply 3: Specimen
All biopsy samples were placed and fixed in formalin, which were processed in paraffin blocks and stained with hematoxylin and eosin in an outside facility in order to eliminate bias and maintain blindness of both pathologists. Each sample was labeled with A, B, or C letter depending on which animal, followed by targeted lobe and then by number of sample. The slides were then provided to two pathologists separately who were blinded to the corresponding biopsy tool. If the sample contained multiple fragments, the number of fragments was recorded and then clumped together in a cell block.

The histologic quality of each sample was assessed using a score that accounted, by order of importance, for area of specimen size, alveolated tissue, size of bronchial tissue, presence of crush artifact and presence of hemorrhage. The large samples with high alveolated tissue/bronchial tissue ratio, and minimal crush artifact and hemorrhage were given a high score. The small sample with low alveolated/bronchial tissue ratio with significant crush artifact and hemorrhage were given a low score. (Table 2)

ADDITIONAL COMMENT: The authors have added a description to Table 2 to make it more detailed and accessible, but please add the following points:
1. Regarding Bronchial tissue
Please clearly indicate the difference between No or scant, No or scant, Present, and Present as you have indicated.
Reply: this is defined in table 2- thank you
2. Please clarify the difference in each of the evaluations of Crush artifact, Hemorrhage as well as the above.
Reply: Crush artifact is artifact caused by biopsy tool and hemorrhage is just evidence of bleeding
3. Since you mentioned the percentage of alveolated tissue, percentage of bronchial tissue, please give us a specific number for that percentage.
Reply: this is defined in table 4 but also attached to this reply the raw data/numbers which we don't see the need to include in the actual manuscript

lung alveolar tissue area (mm^2)

A	B	C
9	2	0
7.5	5	0.25
2	4.5	2
1.5	1	2
2	2.25	2
0	1	0
0	4	1.2
1.2	2.25	0
1	0	0
2.4	0	0
0.75	0	0.25
2	5	0