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Reviewer A

Major comments:

- The manuscript is focused on the use of the 25-gauge TBNA needle. This needle was consistently shown in various studies to be inferior to 21- and 22-gauge needles in terms of acquiring adequate cytology specimens and therefore fell out of favor in many institutions. Indeed, this is reflected in the current manuscript, where in cases 2 and 3, little or no diagnostic tissue was acquired via the 25-G needle TBNA. Prior publications described tract establishment with 21- and 22-G needles. The authors need to clearly justify the choice to use the 25-G needle, beyond its use as a "drill". As currently described, there seem to be no cytolopathologic/clinical or financial justification for the use of this particular needle.

[Response]

Thank you for this insightful comment.

As you have pointed out, the 25-gauge needle is inferior to a thicker needle in terms of specimen collection. In our case, EBUS-TBNA was mainly for tract formation for EBUS-CB rather thanspecimen collection. This is because EBUS-CB can collect a sufficient volume of better quality specimens than EBUS-TBNA. EBUS-CB of central lymph node lesions often uses electrocautery devices and 22-gauge needles; however, these devices may be unavailable for intrapulmonary lesions, as in our case. The use of a 25-gauge needle in such cases may allow successful EBUS-CB; accordingly, we consider this case report as important from this aspect. Moreover, a 25-gauge needle has advantages in terms of flexibility and safety. Although there may be some concern that a 25-gauge needle may not be sufficient to create a tract, at least in our three cases, the forceps and

cryoprobe were easily inserted into the lesion after several EBUS-TBNA punctures. The ability to create a tract into a lesion with a 25-gauge needle is a clinically important finding of our study.

As per similar suggestions by other reviewers, we have included a comparison with 22- and 25-gauge needles in the discussion section (see page 13, line 180 to 187). Specifically, we have indicated that the 25-gauge needle is safer and more flexible than the 22-gauge needle and can be safely tracted in the more peripheral bronchial region.

- Page 13, line 180 to 187, Changes.

There have been reports of tract creation for EBUS-CB using 19- to 22-gauge needles (10,11). However, the 25-gauge EBUS-TBNA needle with high flexibility and safety than 22 gauge or thicker needles (12) allows for safe tracting in the peripheral bronchial area, and may allow for the addition of EBUS-CB in diagnosing intrapulmonary lesions that have not invaded the bronchi or airway. Specifically, although the 25-gauge needle yields a smaller specimen volume than the 22-gauge needle (13), EBUS-TBNA with a 25-gauge needle allows safer tract creation in more peripheral intrapulmonary lesions for EBUS-CB.

- The role of "core" biopsies in mediastinal, hilar, or central lung parenchymal lesions suspicious to represent solid tumors is questionable. Current data shows that cytology material obtained via EBUS-TBNA is in a majority of cases sufficient for diagnostic purposes as well as ancillary studies, such as IHC, DNA-based, and RNA-based assays. All 3 cases described in the current manuscript involve solid cancers. Please provide a concrete rationale/indications that justify obtaining "core" biopsies in these cases from both clinical and financial standpoints.

[Response]

Thank you for this insightful comment. Regarding diagnosis, EBUS-TBNA may be sufficient for malignant diseases; however, the addition of EBUS-CB to EBUS-TBNA has been shown to

improve the suitability of tissue samples for molecular and immunological analysis of NSCLC (PMID:36279880). Therefore, we believe that including EBUS-CB is suitable for facilitating NGS testing or immunostaining, as in our cases. Therefore, we have included the suggested information in the revised manuscript (see pages 15 to 16, lines 220 to 226).

- Pages 15 to 16, line 220 to 226, Changes.

EBUS-TBNA for peripheral intrapulmonary lesions has been shown to have a higher diagnostic yield than endobronchial ultrasound-guided transbronchial biopsy (20). However, previous studies have reported that samples obtained from lymphadenopathy using EBUS-CB are of higher quality than those obtained using EBUS-TBNA (21). Furthermore, compared with EBUS-TBNA alone, EBUS-CB has been shown to improve the suitability of tissue samples for molecular and immunological analysis of NSCLC (22).

- The discussion section would benefit from a major revision. It needs to be abbreviated/ shortened as many ideas are repeated and redundant. Please restructure according to standard scientific composition:

a. Restate research question/hypothesis

b. Summarize key findings

c. Interpret your findings

d. Compare to existing literature

e. Provide limitations

f. Provide implications

g. Provide future directions

[Response]

Thank you for this insightful comment. Accordingly, we have revised the discussion section as per

your suggestion and those of o other reviewers.

Minor comments:

- Introduction, lines 81-83: please provide a citation to support the statement.

[Response]

Thank you for the suggestion. Owing to the rigidity of high-frequency devices, previous studies (PMID:26912301) have only conducted EBUS-IFB of central lesions on lymph nodes. This indicates the limitations of EBUS-guided electrocautery-induced airway incisions, and we have included this information (see page 6, line 73).

- Please indicate if ROSE was used in any/all of the procedures described.

[Response]

Thank you for this comment. ROSE was implemented in all cases. This has been clarified in the revised manuscript (see page 8 to 9, line 99 to 101; page 10, lines 131 to 132; and page 12, lines 153 to 155).

- Page 8 to 9, line 99 to 101, Changes.

The EBUS-TBNA specimen was confirmed as positive through rapid on-site evaluation (ROSE).

- Page 10, line 131 to 132, Changes.

Both EBUS-TBNA and EBUS-CB specimens were positive in the ROSE.

- Page 12, line 154 to 155, Changes.

The EBUS-TBNA specimens tested positive for ROSE.

- Case #1: please clarify what was the final diagnosis. NSCLC with partial squamous features is not a standard classification for NSCLC.

[Response]

Thank you for this insightful comment: The HE staining image was indicative of non-small cell lung cancer. Moreover, although HE staining did not confirm the histology, it presented some features of squamous cell carcinoma. Immunostaining was performed to confirm the diagnosis; however, there were no characteristics of adenocarcinoma or squamous cell carcinoma; accordingly, the final diagnosis was NSCLC NOS. Therefore, we revised the description as indicated (see page 9, lines 110 to 111).

- Page 9, line 110 to 111, Changes.

the patient was diagnosed with NSCLC not otherwise specified

- Case #2: please indicate the type of thyroid cancer the patient had and clarify what was the final case diagnosis.

[Response]

Thank you for raising this important point. The thyroid cancer was a papillary thyroid carcinoma; accordingly, we have corrected the description (see page 9, line 116; page 11, line 138).

- Page 9, line 116 and page 11, line 138, Changes.

papillary thyroid cancer

- Case #3, line 172: please revise "approximately >=30%". What was the actual tumor content?

[Response]

Thank you for your valuable comments. We have revised the description because the actual content

was 30% (see page 12, line 165). The guideline for submitting the Oncomine *Dx Target Test Multi-CDx system* analysis is that the tumor content must be ≥30%, and we had previously stated that to place emphasis on reaching this threshold.

- Page 12, line 165, change.

≥30% ⇒ 30%

- Case #3, line 175: change "was" to "were".

[Response]

Thank you for pointing this out. We apologize for this proofreading error and have made the appropriate correction (see page 12, line 169).

- Page 12, line 169, Change.

was ⇒ were

Reviewer B

-Authors shared 3 cases of 25-gauge needle for tract creation, and mentioned that 25-gauge needle was chosen for its flexibility. Although I have not performed bronchoscopy in above cases, I expect 22-gauge needle might also be a good option in lower lobe intrapulmonary lesions. As 22-gauge needle has bigger lumen than 25-gauge needle, 22-gauge needle could create a tract for EBUS-CB with larger diameter with less effort, thereby enhancing introduction of diagnostic tools for EBUS-CB. The usefulness of 22-gauge needle was previously reported in a study by Kono-Yamamoto et al (Respiration 2023; 102: 143-53). However, as 22-gauge needle is less flexible, it may have limited access to bronchi with acute angle such as upper lobe or superior segment of lower lobe.

Thus, I suggest adding contents on comparison between these two types of needles regarding clinical outcomes, such as lesion location accessibility, tract creation success rate, tract creation time, diagnostic yield, and so on, if possible.

[Response]

Thank you for your insightful comments and suggestions.

As you have stated, we used a 25-gauge instead of a 22-gauge because of its flexibility and safety. The more flexible 25-gauge needle allows us to perform EBUS-TBNA on intrapulmonary lesions in the upper and lower lobes, where the angle of entry s acute. After preparing this case report, we encountered a case where EBUS-TBNA was successfully performed using a 25-gauge needle for an intrapulmonary lesion in the upper lobe that was difficult to puncture using a 22-gauge needle and subsequent EBUS-CB. In Case 3, the pulmonary artery was visualized using EBUS, but a 25-gauge needle was used to perform the puncture more safely. Although there is some concern that a 25-

gauge needle may not create a sufficiently large tract, in all three cases, a sufficiently large tract was successfully created after several EBUS-TBNA procedures and subsequent EBUS-CB. The fact that EBUS-CB could be performed with a 25-gauge needle without any complications is an important point conveyed in this case report. There is also a concern that a 25-gauge needle may not yield sufficient specimens. However, the purpose of EBUS-TBNA with a 25-gauge needle is to create a tract, with the subsequent EBUS-CB yielding a sufficient specimen volume.

Accordingly, we have added a comparison of 22-gauge and 25-gauge needles in the Discussion section (see page 13, line 180 to 187). Specifically, in the Discussion section, we have added that the 25-gauge needle is safer and more flexible than the 22-gauge needle and can be safely tracted in the more peripheral bronchial region, citing supporting papers.

- Page 13, line 180 to 187, Changes.

There have been reports of tract creation for EBUS-CB using 19- to 22-gauge needles (10,11). However, the 25-gauge EBUS-TBNA needle with high flexibility and safety than 22 gauge or thicker needles (12) allows for safe tracting in the peripheral bronchial area, and may allow for the addition of EBUS-CB in diagnosing intrapulmonary lesions that have not invaded the bronchi or airway. Specifically, although the 25-gauge needle yields a smaller specimen volume than the 22-gauge needle (13), EBUS-TBNA with a 25-gauge needle allows safer tract creation in more peripheral intrapulmonary lesions for EBUS-CB.

Reviewer C

-The authors present 3 case studies of EBUS with 25g needle sampling followed by forceps and cryo core biopsies. I am struggling to see the novelty here. They talk about sampling peripheral lung lesions, so are they using a smaller EBUS? The EBUS is mentioned in the case as "the convex probe endobronchial ultrasound (CP-EBUS) scope (BF-104 UC290F; Olympus, Tokyo, Japan)" but the size difference isn't discussed until the last paragraph before the conclusion. If it is the combined use of smaller EBUS + 25g needle, then publishing a 3 patient case series makes sense.

[Response]

Thank you for your insightful remarks.

In all three cases, we used CP-EBUS (UC-290) with a narrow tip. This scope has a narrower tip and greater range of motion than the conventional EBUS scope. This allows penetration into the upper and upper-lower lobes, where the angle of incidence is acute. However, in such areas, it is difficult to use electrocautery or a 22-gauge needle to create a tract because of space limitations. Therefore, we created a tract for EBUS-CB using a 25-gauge needle. This is the first report of EBUS-CB using a 25-gauge needle to create a tract for intrapulmonary lesions beyond the central locations. Our method takes advantage of the flexibility and safety of the 25-gauge needle. Accordingly, we believe that the use of a thinner 25-gauge needle is a significant aspect of this case report.

Therefore, we have added a comparison of 22- and 25-gauge needles and discussed the use of a 25-gauge needle, taking into account the comments of the other reviewers (see page 13, line 180 to 187). Specifically, in the Discussion section, we have added that the 25-gauge needle is safer and more flexible than the 22-gauge needle and can be safely tracted in the more peripheral bronchial region, citing supporting papers. In addition, although a 25-gauge needle can collect a smaller volume of specimen than a 22-gauge needle, the purpose of EBUS-TBNA with a 25-gauge needle

in this case report was to create a tract for EBUS-CB, and that with a 25-gauge needle was to create a tract more safely in more peripheral intrapulmonary lesions.

- Page 13, line 180 to 187, Changes.

There have been reports of tract creation for EBUS-CB using 19- to 22-gauge needles (10,11). However, the 25-gauge EBUS-TBNA needle with high flexibility and safety than 22 gauge or thicker needles (12) allows for safe tracting in the peripheral bronchial area, and may allow for the addition of EBUS-CB in diagnosing intrapulmonary lesions that have not invaded the bronchi or airway. Specifically, although the 25-gauge needle yields a smaller specimen volume than the 22-gauge needle (13), EBUS-TBNA with a 25-gauge needle allows safer tract creation in more peripheral intrapulmonary lesions for EBUS-CB.

Reviewer D

-A well-thought-out perspective. It would be helpful to know if any diagnoses were made based purely on EBUS-TBNA in any of the three cases, and if any immunostaining, and multigene mutation testing was attempted at all. In addition, it would interest the readers to know the post-EBUS-CB results.

[Response]

Thank you for your insightful comments. Accordingly, we have revised and added text in accordance with the reviewer's comments (see page 9, lines 106 to 111, page 12, lines 169 to 171).

In Cases 1 and 2, diagnosis was possible with the EBUS-TBNA specimen; in Case 3, ROSE was positive, but HE staining was difficult to evaluate because of strong necrosis. In all cases, the EBUS-TBNA specimens contained only a small number of tumor cells, making NGS analysis difficult. In addition, since we performed EBUS-CB from the beginning with the expectation that immunostaining and NGS could be performed with high probability, immunostaining and NGS analyses were only attempted on the EBUS-CB specimens. The patient was promptly discharged from the hospital without major complications after the test, which allowed introduction of treatment. The corresponding changes to reflect these points are shown below.

- Page 9, line 106 to 111, Changes.

The EBUS-TBNA specimens showed only a small amount of tumor cells, while the EBUS-CB specimens showed a sufficient amount of tumor cells. Immunostaining of the EBUS-CB specimen was negative for thyroid transcription factor-1, Napsin A, cytokeratin 5/6, p40, chromogranin A, synaptophysin, and cluster of differentiation 56, the patient was diagnosed with NSCLC not otherwise specified.

- Page 12, line 169 to 171, Changes.

No serious complications occurred in any of the cases, and the patients were discharged the day after the examination, allowing prompt introduction of treatment. Furthermore, the scope tip was not damaged in any of the cases.

Reviewer E

-1. The introduction is very informative and addresses the main reasons for the need in TBNA and limitations with electrocautery devices.

[Response]

Thank you for your review. We also appreciate your appreciative comments regarding the introduction.

-2. All cases reflect the advantage of getting a sample by forceps/cryobiopsy than with TBNA and therefore the need for a good method to create a intra-bronchial tract. The cases are beautifully presented and interesting. The authors should add to the last sentence if any complications occurred in the days after the procedures, such as infections or admissions.

[Response]

Thank you for pointing this out. We have included a revision as per your suggestion (see page 12, line 169 to 171).

- Page 12, line 169 to 171, Changes.

No serious complications occurred in any of the cases, and the patients were discharged the day after the examination, allowing prompt introduction of treatment. Furthermore, the scope tip was not damaged in any of the cases.

-3. In our center we use a similar method to create a route for EBUS-CB. However, we repeatedly encounter an issue with finding the exact spot were the tract was performed by the EBUS-TBNA and the correct angle to successfully insert the cryo-probe in the tract. Did you experienced a similar issue? if so, do you have any recommendations for others on how to deal with it?

[Response]

Thank you for pointing this out regarding an important point. Fortunately, we could smoothly insert the forceps and cryoprobes in this case. However, as you have pointed out, insertion is usually difficult. We consider the following points as important for tract creation in EBUS-TBNA: Always advance the needle tip right up to the bronchial mucosa, lifting it slightly with each pass. Ensure that TBNA is consistently performed from the same puncture hole. During EBUS-TBNA, gradually apply up/down angulation and slight left/right rotation to expand the puncture hole. In cases in which it is challenging to insert cryoprobes or forceps, additional punctures during TBNA may be necessary. If there is some resistance, forceps can be used to grasp the epithelium or the assistant can push the scope simultaneously with cryoprobe insertion, which can also be effective. We have added this information to the Discussion section (see page 14, line 203-206).

- Page 14, line 203-206, Changes.

In all of the present cases, forceps or cryoprobe could be smoothly inserted after EBUS-TBNA; however, difficult insertion can be mitigated by additional EBUS-TBNA, biting the insertion site with forceps, or a second surgeon pushing the scope during insertion could facilitate forceps or cryoprobe insertion.

-4. The authors should add some examples from prior research in this field. Are any prior studies assessed the use of TBNA in these cases? Where any review performed on this field?

[Response]

Thank you for this insightful comment. Although there are only a few reports, there have been reports of EBUS-CB using needles larger than 22-gauge, which has been indicated in the revised manuscript (see page 13, line 180 to 181).

- Page 13, line 180-181, Changes.

There have been reports of tract creation for EBUS-CB using 19- to 22-gauge needles (10,11).

-5. The choice between forceps and cryobiopsy after use of TBNA is crucial to successfully obtain large-enough tissue for histology. I think the authors should address this topic in the discussion, with the advantages for each technique. In this regard, the authors could use the following paper to support the use of cryobiopsy, that describe its use compared with forceps-biopsy, and showed a better diagnostic yield with only higher rate of bleeding that did not result in major complications:

https://pubmed.ncbi.nlm.nih.gov/37634496/

[Response]

Thank you for this interesting suggestion. Accordingly, we have included it to the discussion (see page 17, line 250 to 254).

- Page 17, line 250 to 254, Changes.

Compared with forceps biopsy, cryobiopsy has been shown to have better diagnostic results without major complications(25). Accordingly, EBUS-CB using a 1.1mm cryoprobe with a thinner CP-EBUS scope that has a narrow 5.9mm tip may facilitate the diagnosis of more peripheral intrapulmonary lesions.

Reviewer F

-Remarks:

In the title, I suggest adding the word 'convex' in front of 'endobronchial ultrasound-guided core biopsy.' Since most EBUS-guided core biopsies are performed with radial-EBUS guidance, clarifying the use of the convex EBUS probe would prevent confusion.

[Response]

Thank you for pointing this out very important point. Adding 'convex' to the title would certainly convey our message more clearly.

We modified our title as advised (see page 1, line 1).

- Title, Page 1, line 1, Change.

Tract creation with a 25-gauge needle for convex endobronchial ultrasound-guided core biopsy in intrapulmonary lesions adjacent to bronchi: a case report

-Discussion:

Page 6, Line 192: I suggest revising this sentence to be less robust. The diagnostic yield of other bronchoscopic techniques may have limitations but are not generally considered 'inadequate.'

[Response]

Thank you for the suggestion. I have changed "inadequate" to "limited" based on your suggestion to convey a more correct nuance (see page 14, line 190 to 194).

- Page 14, line 190 to 194, Changes.

The diagnostic yield of conventional bronchoscopy performed using forceps, aspiration

needles, and cryoprobes has been limited for diagnosing intrapulmonary lesions adjacent to the segmental and subsegmental bronchi owing to the requirement for collecting tumor cells beyond the bronchial wall and the absence of real-time echo guidance (14-17).

-Page 7, Line 242: Why is a 22-gauge needle unavailable to use with the thin CP-EUBS scope in situations where a 1.1mm cryoprobe is accessible? Clarification would be needed on the inability to use a 22-gauge needle.

[Response]

We thank you for pointing this out to us.

In this discussion, we mention the thinner CP-EBUS scope with a 5.9-mm tip under development. This new scope has a 1.7-mm working channel, which is narrower than that of the conventional BF-UC290F scope (2.2-mm working channel) we used this report. Therefore, a 22-gauge EBUS-TBNA needle with a diameter of 1.9-mm mm not be attached to this new scope. The method we report here, which uses a 25-gauge needle, overcomes this limitation. To better convey the above, we have added a description of the working channel of the scope, as well as a distinction between the thin CP-EBUS scope and the thinner CP-EBUS scope. Specifically, the phrase "because of the small 1.7-mm working channel of the scope" was added. In addition, "thinner" was added to separate "CP-EBUS" and "thinner CP-EBUS" (see page 16 to 17, line 243 to 250).

- Page 16 to 17, line 243 to 250, Changes.

Notably, a thinner CP-EBUS scope with a 5.9-mm tip is currently under development (24), which can be inserted up to the fourth-generation bronchus, but can only fit up to a 25-gauge needle given the small 1.7-mm working channel of the scope. However, by performing EBUS-TBNA with a 25-gauge needle for tract creation, as in the present case report, the thinner CP-EBUS scope may enable clinicians to perform subsequent EBUS-CB with a 1.1-mm cryoprobe

of more peripheral peribronchial lesions and overcome the inability to use a 22-gauge needle.

-Can the authors add some explanations on why they used the 25-gauge needle instead of 19 to 22-gauge needles, which are usually used to perform EBUS-TBNA or EBUS-guided needle biopsy?

[Response]

Thank you for your remarks regarding the importance of using 25-gauge needles instead of 22-gauge needles. This point has been raised by the other reviewers.

As you have stated, we used a 25-gauge instead of a 22-gauge because of its flexibility and safety. The more flexible 25-gauge needle allows us to perform EBUS-TBNA on intrapulmonary lesions in the upper and lower lobes, where the angle of entry s acute. After preparing this case report, we encountered a case where EBUS-TBNA was successfully performed using a 25-gauge needle for an intrapulmonary lesion in the upper lobe that was difficult to puncture using a 22-gauge needle and subsequent EBUS-CB. In Case 3, the pulmonary artery was visualized using EBUS, but a 25-gauge needle was used to perform the puncture more safely. Although there is some concern that a 25-gauge needle may not create a sufficiently large tract, in all three cases, a sufficiently large tract was successfully created after several EBUS-TBNA procedures and subsequent EBUS-CB. The fact that EBUS-CB could be performed with a 25-gauge needle without any complications is an important point conveyed in this case report. There is also a concern that a 25-gauge needle may not yield sufficient specimens. However, the purpose of EBUS-TBNA with a 25-gauge needle is to create a tract, with the subsequent EBUS-CB yielding a sufficient specimen volume.

Accordingly, we have added a comparison of 22-gauge and 25-gauge needles in the Discussion section (see page 13, lines 180 to 187). Specifically, in the Discussion section, we have added that the 25-gauge needle is safer and more flexible than the 22-gauge needle and can be safely tracted in the more peripheral bronchial region, citing supporting papers.

- Page 13, line 180 to 187, Changes.

There have been reports of tract creation for EBUS-CB using 19- to 22-gauge needles (10,11). However, the 25-gauge EBUS-TBNA needle with high flexibility and safety than 22 gauge or thicker needles (12) allows for safe tracting in the peripheral bronchial area, and may allow for the addition of EBUS-CB in diagnosing intrapulmonary lesions that have not invaded the bronchi or airway. Specifically, although the 25-gauge needle yields a smaller specimen volume than the 22-gauge needle (13), EBUS-TBNA with a 25-gauge needle allows safer tract creation in more peripheral intrapulmonary lesions for EBUS-CB.

Reviewer G

-The text is well written in English, clear and precise.

Topics are clearly stated, as are the techniques used to perform maneuvers in endoscopy.

The topic is very interesting and very current in the field of interventional pulmonology.

Because there are no standardized procedures for performing this type of sampling, any new technique that is successfully demonstrated is worthy of attention.

Of course, the paper is not conclusive about the issue, but it helps to input new information into this area.

[Response]

Thank you for your peer review. We are pleased to receive your gratifying comments. We believe that this novel technique has the potential to make it easier for many respiratory physicians to perform EBUS-CB. Of course, as you say, this is not definitive, but we believe that it is important to introduce such cases.