Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-23-1598

Reviewer A

The authors retrospectively reviewed the benefit of additional TBNA using the PeriView FLEX needle combined with EBUS-GS to diagnose peripheral pulmonary nodules.

Comment 1:
Abstract
As the authors mentioned, we cannot conclude that an additional rEBUS-GS TBNA improves the diagnostic yield. When the lesion was visualized as within EBUS-GS, there was no additional diagnostic value by TBNA.
Reply:
Thanks for your comment. Although we think the increase in overall diagnostic yield from 63.5% (47/74) to 70.3% (52/74) is considered meaningful, your point that the increase in diagnosis yield only occurs in a certain situation (probe adjacent to the lesion) is reasonable. So we think “can be” should modified to more non-conclusive wording like “might be”. We have modified our text as follows.

Changes in the text (blue):
Abstract (see page 3, line 51)
Conclusions: In rEBUS GS procedures for PLLs, the diagnostic yield might be improved by implementing TBNA in addition to TBLB. In particular, additional TBNA is preferable if the probe is adjacent to the lesion rather than within the lesion on rEBUS.

Discussion (see page 13, line 295)
In conclusion, when performing the rEBUS GS procedure for tissue biopsy of peripheral lung lesions, if the lesion is adjacent, the diagnostic yield might be increased by adding rEBUS GS TBNA using the PeriView FLEX TBNA needle in addition to rEBUS GS TBLB.

Comment 2:
Introduction
Medical thoracoscopy is not used for the diagnosis of peripheral pulmonary nodules. Recently, robotic bronchoscopy has become a powerful tool for treating peripheral pulmonary nodules.
Reply:
Thanks for your comment. In accordance with your point, in the revised manuscript, the description of “medical thoracoscopy” was deleted and “robotic bronchoscopy” was added, as follows.

Changes in the text (blue):
Introduction (see page 4, line 69~)
The preferred initial site for the tissue biopsy should be easily and safely accessible, and the approach may vary depending upon experience and available facilities (5). For peripheral lung lesions, it can be selected from transthoracic needle biopsy (TTNB) or special bronchoscopic

procedures (using electromagnetic navigation bronchoscopy, radial endobronchial ultrasound [rEBUS], or robotic bronchoscopy) (5,6).

Discussion (see page 10, line 230~)
...rEBUS is very safe (pneumothorax, 0–3%; hemoptysis, 0–1%), but its diagnostic yield is relatively low (60–70%) compared to that of TTNB (7,16). In terms of the robotic bronchoscopy, while the authors have yet to gain firsthand experience, research suggests a higher diagnostic yield (around 80%) compared to rEBUS or electromagnetic navigation bronchoscopy (6). However, its availability is limited due to the high setup costs (17).

Comment 3. Methods
The critical issue of this manuscript is the way of patient selection. The authors only selected the patients who underwent rEBUS-GS TBNA and rEBUS-GS TBLB. In this situation, the authors only evaluated the patients who required additional procedures to conventional rEBUS-GS TBLB; hence, it was natural that the additional TBNA procedure produced the additional diagnostic value. However, this effect might be caused by the selection bias.
Reply:
Thanks for your comment. Given the nature of retrospective studies, we think that selection bias is unavoidable. We seek your generous understanding on this matter. It is not possible to conduct all research prospectively. We believe that after exploring the possibilities through retrospective studies, it is possible to consider conducting prospective studies. In the revised manuscript, the limitation section of the discussion describes the presence of selection bias and the need for prospective studies as follows.

Changes in the text (blue):
Discussion (see page 13, line 287~)
The present study had some limitations. First, this was a retrospective, single-institution study with a small sample size, which may have had various sources of selection bias that were not identified or controlled. In particular, the number of patients diagnosed using rEBUS GS TBNA was small. Second, the time required for additional rEBUS GS-TBNA, which is a quality assessment factor, was not measured. In these regards, our results should be reaffirmed through a prospective study with a larger sample.

Comment 4. Methods
Did the authors use rapid on-site evaluation during the procedure?
Reply:
No, we did not. For the readers, this is explained as follows.

Changes in the text (blue):
Methods (see page 6, line 131~)
...At least two needle passes were performed until core tissue was obtained, with more than 50 to-and-fro needle agitations per pass under 20 ml of negative pressure. The core tissue was separated and fixed with formalin and send for the histopathology and molecular analysis (13). Rapid on-site evaluation of pathological specimens was not conducted. All procedures were performed with the

help of fluoroscopy under conscious sedation. A routine chest radiograph was obtained within 2 hours of the end of the procedure.

Comment 5. Results
Were there any undiagnosed cases of rEBUS-GS TBNA but diagnosed by rEBUS-GS TBLB?
Reply:
Please see Figure 1. Sixteen cases were not diagnosed by rEBUS GS TBNA but diagnosed by rEBUS GS TBLB.

Comment 6. Discussion
When the authors mentioned the additional cost for ancillary modality, they also had to explain the extra fee for using PeriView FLEX. Furthermore, upfront surgery with a frozen section during surgery may be the most cost-effective strategy for the small peripheral nodule.
Reply:
Thanks for your comments.

Firstly, we did not mention the additional cost for ancillary modality; we only discussed the cost-effectiveness of radial EBUS compared to electromagnetic navigation bronchoscopy (see page 10, line 226). The extra fee for the PeriView FLEX needle (Olympus, Tokyo, Japan) is around 200,000 Korean Won (approximately 150 USD). However, considering the context of the manuscript, it seems very unnatural to disclose the price for the PeriView FLEX needle alone. Therefore, we decided not to include pricing information in the manuscript. We ask for your generous understanding.

Secondly, we agree with your opinion that upfront surgery with a frozen section may be “the most cost-effective strategy” for the small peripheral nodule. We revised the manuscript as follows in the introduction section. Considering the context, that was mentioned in the introduction section rather than the discussion section. We ask for your generous understanding regarding this.

Changes in the text (blue):
Introduction (see page 4, line 63)
The discovery of a lung lesion prompts a decision regarding observation, direct surgery, or tissue biopsy (1-3). If the identified lesion is located on the periphery and is small (i.e., a solid nodule < 15 mm or a part-solid nodule with a solid component < 6–8 mm), the patient can be placed under observation (3). However, if the lesion is peripheral but of considerable size (i.e., a solid nodule of 15–30 mm or a part-solid nodule with solid component ≥ 6–8 mm) or growing, direct surgery is desirable and most cost-effective unless a distant or nodal metastasis is suspected (3,4).

Comment 6. Discussion
TBNA usually provides the cytological material for the diagnosis. What did the authors think about this limitation?
Reply:
Thanks for your comments. Through TBNA, not only liquid samples but also core tissue can be obtained. The core tissue can be formalin-fixed using cell block technique to enable sufficient

histopathological and molecular diagnosis. This has been proven by researches [J Bronchology Interv Pulmonol. 2019 Oct;26(4):237, PLoS One. 2022 Feb 2;17(2):e0263342, Cancer Cytopathol. 2020 May;128(5):333, Diagnostics (Basel). 2021 Dec 10;11(12):2331] and is currently used around the world.

In the revised manuscript, the specimen processing procedure has been included along with a reference to aid the reader's understanding, as follows.

Changes in the text (blue):
Methods (see page 6, line 129~)
rEBUS GS TBLB was performed by alternating cycles of one brush and two biopsies through the GS with a GS-dedicated cytology brush (BC-204D-2010, Olympus, Tokyo, Japan) and biopsy forceps (FB-233D, Olympus, Tokyo, Japan); this process was repeated ≥ 3 times until at least four biopsy specimens were obtained. rEBUS GS TBNA was performed through the GS using a PeriView FLEX TBNA needle. At least two needle passes were performed until core tissue was obtained, with more than 50 to-and-fro needle agitations per pass under 20 ml of negative pressure. The core tissue was separated and fixed with formalin and send for the histopathology and molecular analysis (13).

Comment 7. Discussion
Did the authors evaluate the additional diagnostic value by TBNA based on the tumor location?
Reply:
Please see the Table 4. As you can see, there was a trend toward higher diagnostic yield of rEBUS GS TBNA in the upper lobes [Location of PLL, n (%): Right upper lobe, 1 (20.0); Left upper lobe, 4 (80.0)], but this did not reach statistical significance. No additional revision was made on this in the manuscript. We ask for your understanding.

Comment 8. Discussion
How can we know “when the lesion is too difficult to open with the rEBUS GS TBLB forceps”?
Reply:
Apologies for the insufficient explanation. This process can be seen by looking at fluoroscopy. All our procedures were done under the help of the fluoroscopy. To help readers understand, the manuscript has been revised as follows.

Changes in the text (blue):
Methods (see page 7, line 132~)
...At least two needle passes were performed until core tissue was obtained, with more than 50 to-and-fro needle agitations per pass under 20 ml of negative pressure. The core tissue was separated and fixed with formalin and send for the histopathology and molecular analysis (13). Rapid on-site evaluation of pathological specimens was not conducted. All procedures were performed with the help of fluoroscopy under conscious sedation. A routine chest radiograph was obtained within 2 hours of the end of the procedure.

Discussion (see page 13, line 284)

...Therefore, in the case of within-type peripheral lung lesions on rEBUS, only rEBUS GS-TBLB was deemed sufficient. Our multivariate analysis (within-type rEBUS visualization was the only independently associated factor for rEBUS GS-TBLB diagnosis) supported these results. After confirming our results, rEBUS GS TBNA has not generally been performed in within-type peripheral lung lesions at our institution, except when the lesion is too difficult to open with the rEBUS GS TBLB forceps (on fluoroscopy).

Reviewer B

The authors present a retrospective study of the use of a Peri-view flex needle w rpEBUS guided bronchoscopy and compare the needle to transbronchial biopsies.

Suggested revisions:

Comment 1. Introduction. The first sentence states the discovery of a lung lesion prompts a decision regarding "observation or direct surgery." This paper is specifically about biopsy so perhaps they should mention tissue biopsy as a next course of action for a nodule.
Reply:
Thank you for your comment. We agree with you. In the revised manuscript, the sentenced was modified as follows.

Changes in the text (blue):
Introduction (see page 4, line 59)
The discovery of a lung lesion prompts a decision regarding observation, direct surgery, or tissue biopsy (1,2).

Comment 2. The next five sentences which discuss the decision-making around when to pursue biopsy do not have any references. This section needs references.
Reply:
Thank you for your comment and assistance. In the revised manuscript, we added references as follows.

Changes in the text (blue):
Introduction (see page 4, line 61~)
The discovery of a lung lesion prompts a decision regarding observation, direct surgery, or tissue biopsy (1-3). If the identified lesion is located on the periphery and is small (i.e., a solid nodule < 15 mm or a part-solid nodule with a solid component < 6–8 mm), the patient can be placed under observation (3). However, if the lesion is peripheral but of considerable size (i.e., a solid nodule of 15–30 mm or a part-solid nodule with solid component ≥ 6–8 mm) or growing, direct surgery is desirable and most cost-effective unless a distant or nodal metastasis is suspected (3,4). Except for the above situations, such as in cases the nodule (tumor) is > 30 mm, a central lesion, multiple lesions, distant metastasis, or visible lymphadenopathy on imaging studies, or the possibility of benign disease that can be treated medically, a tissue biopsy will eventually be required (3,5). The

preferred initial site for the tissue biopsy should be easily and safely accessible, and the approach may vary depending upon experience and available facilities (5). For peripheral lung lesions, it can be selected from transthoracic needle biopsy (TTNB) or special bronchoscopic procedures (using electromagnetic navigation, radial endobronchial ultrasound [rEBUS], or robotic bronchoscopy) (5,6).

Comment 3. The last two sentences in the first introductory paragraph state that medical thoracoscopy, conventional bronchoscopy, and convex EBUS are considered first in that order; however, the order in which these very different procedures are selected depends on the cancer stage, so I find this statement does not seem reasonable.
Reply:
Thanks for your comment. In accordance with your point, we revised the last two sentences as follows: We have shifted our focus to the approach to peripheral lung lesions, omitting the terms "medical thoracoscopy, conventional bronchoscopy, and/or convex endobronchial ultrasound." In addition, we have included a mention of robotic bronchoscopy, as requested by another reviewer.

Changes in the text (blue):
Introduction (see page 4, line 67~)
The discovery of a lung lesion prompts a decision regarding observation, direct surgery, or tissue biopsy (1-3). If the identified lesion is located on the periphery and is small (i.e., a solid nodule < 15 mm or a part-solid nodule with a solid component < 6–8 mm), the patient can be placed under observation (3). However, if the lesion is peripheral but of considerable size (i.e., a solid nodule of 15–30 mm or a part-solid nodule with solid component ≥ 6–8 mm) or growing, direct surgery is desirable and most cost-effective unless a distant or nodal metastasis is suspected (3,4). Except for the above situations, such as in cases the nodule (tumor) is > 30 mm, a central lesion, multiple lesions, distant metastasis, or visible lymphadenopathy on imaging studies, or the possibility of benign disease that can be treated medically, a tissue biopsy will eventually be required (3,5). The preferred initial site for the tissue biopsy should be easily and safely accessible, and the approach may vary depending upon experience and available facilities (5). For peripheral lung lesions, it can be selected from transthoracic needle biopsy (TTNB) or special bronchoscopic procedures (using electromagnetic navigation, radial endobronchial ultrasound [rEBUS], or robotic bronchoscopy) (5,6).

METHODS
Comment 4. rpEBUS GS procedure -- In this section the authors discuss brushing, followed by biopsies. Nowhere in the results are the brushing yields mentioned. The authors will need to address that in the outcomes even though it is not the focus of this paper.
Reply:
Thanks for your comment. In the revised manuscript, we added a new table named “Supplementary Table 1. Diagnosis yields according to each sampling modality”. Additionally, it was briefly addressed in the result section as follows. Please understand that this is not the main focus of this paper, so it was added as a Supplementary Table.

Changes in the text (blue):

Results (see page 8, line 177~)
Diagnostic yield
Among the 74 peripheral lung lesions, 47 (63.5%) were successfully diagnosed using rEBUS GS TBLB. Of the 27 peripheral lung lesions not diagnosed using rEBUS GS TBLB, five (18.5%) were further diagnosed using rEBUS GS TBNA: adenocarcinoma (n = 2), non-small cell lung cancer not otherwise specified (n = 2), small cell lung cancer (n = 1). Additional TBNA increased the diagnostic yield by 6.8% (5/74), resulting in an overall diagnostic yield of 70.3% (52/74) (Figure 1 and 2A). Diagnostic yields for each modality (including biopsy forceps, brushing, or washing etc.) are shown in Supplementary Table 1.

Supplementary Table 1 (see the Supplements Appendix)

Discussions
Comment 5. In the second paragraph, the authors referred to electromagnetic navigation bronchoscopy, however, do not include robotic bronchoscopy, which has been out for three years now. Please mention robotic bronchoscopy (higher yield than EMN) with references and it would be reasonable to mention the cist in hardware and software purchases that limit its availability.
Reply:
Thanks for your comment. In accordance with your point, the revised manuscript added “robotic bronchoscope” and mentioned that its use is limited due to price issues, as follows.

Changes in the text (blue):
Discussion (see page 10, line 223~)
Among the various techniques for biopsy of peripheral lung lesions, TTNB has the highest diagnostic yield (approximately 90%) (5,14) and the highest complication rate (pneumothorax, 15–20%; and hemoptysis, 1–5%) (15,16). Special bronchoscopic procedures such as electromagnetic navigation bronchoscopy, rEBUS, or robotic bronchoscopy have been developed for safer tissue biopsies. rEBUS is more difficult to learn than electromagnetic navigation bronchoscopy and requires a longer training time, but has the advantage of being more cost effective than electromagnetic navigation bronchoscopy despite a similar diagnostic yield (7). We used both rEBUS and electromagnetic navigation bronchoscopy, but recently started using only rEBUS because of cost issues. rEBUS is very safe (pneumothorax, 0–3%; hemoptysis, 0–1%), but its diagnostic yield is relatively low (50–70%) compared to that of TTNB (7,16). In terms of the robotic bronchoscopy, while the authors have yet to gain firsthand experience, research suggests a higher diagnostic yield (around 80%) compared to rEBUS or electromagnetic navigation bronchoscopy (6). However, its availability is limited due to the high setup costs (17).

Comment 6. In paragraph five, the authors mention cryobiopsy and that the scope must be removed at the same time, adding difficulty and risk of bleeding. Please note that there is a 1.1 cryo-probe currently on the market by Erby, which can go through a guide sheath, so this statement does not reflect current available technology. You could certainly mention cost as a limiting factor to using this unit.
Reply:

Thanks for your comment. You are right. The 1.1 mm cryoprobe (ERBE, Germany) has a dedicated Oversheath, which allows repeated cryobiopsy without removing the scope. However, the size of the Oversheath itself is 2.6 mm, making it unsuitable for the thin bronchoscope (outer diameter, 4.0–4.2 mm; working channel, 2 mm) that we use for peripheral lung lesions. For this reason, if you look at a recent paper using cryobiopsy and thin bronchoscope (Cancer Res Treat. 2023;55(2):506), it is written that the scope and cryoprobe were removed together.

In the revised manuscript, the above information was added and the cost issue of cryobiopsy was also mentioned, as follows. We ask for your generous understanding.

Changes in the text (blue):
Discussion (see page 11, line 252~)
Although both conventional transbronchial biopsy and cryobiopsy are good adjunct modalities for rEBUS TBLB with GS, they have inevitable technical drawbacks. Regarding additional conventional transbronchial biopsy, the procedure should be performed after the removal of the GS. When using the GS, an accurate biopsy is possible within or directly in front of the lesion; however, without the GS, the forceps may not reach the lesion accurately. In cases of additional cryobiopsy, the procedure through GS is possible if a 1.1 mm Cryoprobe is used, but the scope must be removed at the same time unless a dedicated oversheath (outer diameter 2.6mm) is used, which is impossible with a thin bronchoscope (outer diameter, 4.0–4.2 mm; working channel, 2 mm); therefore, repeat procedures may be difficult, and the risk of bleeding is relatively high. The cost of cryobiopsy is also considerable.

Reviewer C

Comment 1. put brief section in text about fact there were no complications in either group
Reply:
Thanks for your comment. In the revised manuscript, we added a brief comment addressing complications as follows.

Changes in the text (blue):
Results (see page 8, line 167~)
During the study period, rEBUS GS was performed on 124 peripheral lung lesions from 123 patients. Of these, TBLB and TBNA were used for 74 lesions (73 patients) (Figure 1). The lesions showed the following characteristics: lesion size (mean ± SD), 24 ± 12 mm; nature (solid vs. subsolid), 59 (79.7%) vs. 15 (20.3%); distance from pleura (mean ± SD), 14 ± 14 mm; rEBUS visualization type (probe within peripheral lung lesion vs. probe adjacent to peripheral lung lesion), 56 (75.7%) vs. 18 (24.3%). In terms of complications, no significant incidents of bleeding or pneumothorax were observed in either group (Table 1). The final diagnoses of all peripheral lung lesions are shown in Table 2.

Comment 2. Clarifications about technique
- Was suction used with the periview flex needle?
Reply:

Sorry for the insufficient explanations. We added the information of negative pressure as follows.

Changes in the text (blue):
Methods (see page 6, line 129~)
... At least two needle passes were performed until core tissue was obtained, with more than 50 to-and-fro needle agitations per pass under 20 ml of negative pressure. The core tissue was separated and fixed with formalin and send for the histopathology and molecular analysis, while liquid specimens underwent liquid-based cytopathology (13).

- Was II used when doing needle passes?
Reply:
Sorry for the insufficient explanations. Information regarding the use of fluoroscopy has been added as follows.

Changes in the text (blue):
Methods (see page 7, line 132)
…Rapid on-site evaluation of pathological specimens was not conducted. All procedures were performed with the help of fluoroscopy under conscious sedation. A routine chest radiograph was obtained within 2 hours of the end of the procedure.

- Was rapid onsite cytology present for cases?
Reply:
Sorry for the insufficient explanations. Information regarding rapid on-site evaluation has been added as follows.

Changes in the text (blue):
Methods (see page 6, line 131)
… Rapid on-site evaluation of pathological specimens was not conducted. All procedures were performed with the help of fluoroscopy under conscious sedation. A routine chest radiograph was obtained within 2 hours of the end of the procedure.

Comment 3. How many operators?
Reply:
Sorry for the insufficient explanations. Information regarding the number of operators has been added as follows.

Changes in the text (blue):
Methods (see page 6, line 112~)
All patients underwent a high-quality CT scan (256-MDCT scanners: Somatom Definition AS+ and Somatom Definition Flash; Siemens Healthcare, Forchheim, Germany) before rEBUS to enable hand-drawing of a bronchial map for a target peripheral lung lesion. Two bronchoscopists (JH Kim and T Lee) performed EBUS GS as previously described (7). First, a thin bronchoscope (outer diameter, 4.0–4.2 mm, BF-P260F or BF-P290, Olympus, Tokyo, Japan) was inserted as far as possible into the bronchus nearest to the peripheral lung lesion...

What were the diagnoses of the 5 cases that were diagnosed with TBNA only (non diagnostic on tblbx)

Reply:

Sorry for the insufficient explanations. Information regarding the 5 cases diagnosed with TBNA only has been added as follows.

Changes in the text (blue):

Results (see page 8, line 174~)

Diagnostic yield

Among the 74 peripheral lung lesions, 47 (63.5%) were successfully diagnosed using rEBUS GS TBLB. Of the 27 peripheral lung lesions not diagnosed using rEBUS GS TBLB, five (18.5%) were further diagnosed using rEBUS GS TBNA: adenocarcinoma (n = 2), non-small cell lung cancer not otherwise specified (n = 2), small cell lung cancer (n = 1). Additional TBNA increased the diagnostic yield by 6.8% (5/74), resulting in an overall diagnostic yield of 70.3% (52/74) (Figure 1 and 2A). Diagnostic yields for each modality (including biopsy forceps, brushing, or washing etc.) are shown in Supplementary Table 1.

Comment 4. What modality was used to make the diagnoses of cases that were undiagnosed with TBLB/TBNA?

Reply:

Thanks for your comment. The modalities for the TBLB/TBNA-undiagnosed cases were already addressed as follows (red).

Methods (see page 7, line 142)

Baseline data collection and final diagnosis

Baseline characteristics (age, sex, peripheral lung lesion size [the average of the long and short axes], peripheral lung lesion distance from the pleura, lobar location of the peripheral lung lesion, bronchus sign [presence of an open bronchus connected from a proximal airway in the peripheral lung lesion], and nature of the peripheral lung lesion on chest CT) of all enrolled patients were collected. Final diagnoses were established based on the pathological results. If not diagnosed using rEBUS, further examination (TTNB or surgical resection) was performed.

Comment 5. How can you explain why, when the GS was within the lesion, did the TBNA not produce any positive diagnoses ?(refer table 4 - this seems counterintuitive).

Reply:

Thanks for your comment. In the case of within-type lesions, we think this is because the site of tissue through TBNA is the same location where rEBUS GS TBLB was performed. For this reason, if TBLB is negative, TBNA appears to be equally negative. In the case of adjacent-type lesions, TBNA tissue collection occurs in a different location from where TBLB was performed, so TBNA might be positive even if TBLB is negative.

In the revised manuscript, the above information was added as follows

Changes in the text (blue):

Discussion (see page 12, line 276~)
In comparison, the additional rEBUS GS TBNA using the PeriView FLEX TBNA needle was not helpful for within-type lesions on rEBUS. In our study, additional rEBUS GS TBNA did not at all increase the diagnostic yield of within-type peripheral lung lesions on rEBUS. It is though that, in the case of within-type peripheral lung lesions on rEBUS, performing additional rEBUS GS TBNA may yield tissue samples from the same location where rEBUS GS TBLB was performed. Therefore, in the case of within-type peripheral lung lesions on rEBUS, only rEBUS GS-TBLB was deemed sufficient...

Comment 6. Page 8 line 162 - I think should be "For the 27 rEBUS GS TBLB - undiagnosed cases....."
Reply:
Thank you so much for generously pointing out our mistake. We revised the manuscript as follows.

Changes in the text (blue):
Results (see page 9, line 196)
Subgroup analysis for the rEBUS GS TBLB-undiagnosed group
For the 27 rEBUS GS TBLB-undiagnosed cases, the rEBUS GS TBNA-diagnosed subgroup (n = 5) and the rEBUS GS TBNA-undiagnosed subgroup (n = 22) were compared in a subgroup analysis. TBNA diagnosis was significantly associated with adjacent-to-type EBUS visualization (100.0% [5/5] vs. 45.5% [10/22], P = 0.047) (Table 4).