Peer Review File

Reviewer A

Nice and very well-written retrospective study on EMN-TTNB. There are some minor issues to be solved in a revised version of the manuscript:

Comment 1:

1) INTRODUCTION, line 95: Please, add that bronchoscopic biopsy may be favored not only in centrally located lesions, but also in peripheral lesions when a positive bronchus sign is present.

Reply 1: Thank you for this insightful comment. We have revised the text as the reviewer suggested. (see Page 6, lines 96-98).

Changes in the text: The number of adverse events with PCNB is significantly higher than that with bronchoscopic sampling; therefore, *bronchoscopic biopsy may be favored not only in centrally located lesions, but also in peripheral lesions when a positive bronchus sign is present* (7).

Comment 2:

2) METHODS: Please, describe the safe measures (SpO2 monitoring? ECG? Blood pressure?). Furthermore, information is missing on starvation period before intervention and oxygen supply.

Reply 2: We sincerely appreciate this comment. We have added more information about the safety measure, starvation period, and oxygen supply as suggested (see Pages 8-9, lines 143-147).

Changes in the text: *The standard monitoring consisted of non-invasive blood pressure, heart rate, electrocardiogram, pulse oximetry, and respiratory rate. In order to avoid desaturation episodes, oxygen support provided by nasal prongs was usually required during and after the procedure. Furthermore, a fasting duration of at least 4 hours was recommended before the procedure.*

Comment 3:

3) METHODS: Please, add exclusion criteria/contraindications for the procedure (for example, anticoagulation).

Reply 3: Thank you for this insightful and important comment. We have further described exclusions and contraindications. We have also added relevant references. (see Page 8, lines 129-134).

Changes in the text: *Patients who were unable to express their opinions and did not cooperate due to cognitive impairment were excluded. Prior to the procedure, recent platelet count and coagulogram were checked to rule out coagulation abnormalities. Anticoagulants and antiplatelet agents were discontinued before EMN-TTNB referring to the published guideline (13). Clopidogrel and aspirin were discontinued for 5 days and new oral anticoagulants (NOACs) for 1-4 days considering the type and renal function.*

Comment 4:

4) METHODS: Information on statistical methods is missing.

Reply 4: Thank you for this comment. Information on statistical methods has already been described at the end of the methods section. The contents are as follows. We have also changed 'diagnostic yield' to 'diagnostic accuracy' based on the comments from Reviewer B. (see Page 11, lines 196-209).

Statistical analysis

Categorical variables were described as simple proportions (%), and continuous variables were described as means with standard deviation (SD) *or median (interquartile range [IQR]).* The diagnostic *accuracy* of EMN-TTNB was calculated as the sum of true-positives and true-negatives divided by the total number of cases, excluding the indeterminate cases. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated. The patients who initially had negative results with insufficient information or follow-up duration to conclude as true- or false-negative status were included in the outcome analysis,

assuming all as true-negative or false-negative cases, respectively. This method provided low and high estimates of the diagnostic *accuracy*, sensitivity, specificity, and PPV and NPV of EMN-TTNB for diagnosing malignancy.

Univariate analysis was performed using logistic regression models to determine factors that increase diagnostic *accuracy*. A P-value of <0.05 was considered statistically significant. All statistical analysis was conducted using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA).

Comment 5:

5) RESULTS, line 259: Please say "None of the factors was associated with diagnosis rate rather than positive correlation.

Reply 5: Thank you for this suggestion. We have added the sentence in the revised manuscript as advised (see Page 14, lines 266-267).

Changes in the text:

Factors associated with high diagnostic accuracy

Table 3 shows the univariate analysis of factors related to the high diagnostic *accuracy* of EMN-TTNB. Diagnostic *accuracy* using EMN-TTNB did not show any association with lesion size, distance from the pleura, procedure position, and number of core biopsies obtained. *None of the factors was associated with diagnosis rate.*

Comment 6:

6) DISCUSSION, line 310: 1.6% rather than 16%

Reply 6: Thank you for this comment. We apologize for our mistake. We have corrected it (see Page 16, line 312).

Changes in the text:

Minor bleeding (2/129, 1.6%), chronic obstructive pulmonary disease (COPD) exacerbation (1/129, 0.78%), transient hypercapnia (2/129, *1.6%*) and transient hypoxemia (1/129, 0.78%) were encountered.

Comment 7:

7) DISCUSSION, line 315-323: What about distance to pleura and positive pressure ventilation, which could also attribute to PTX risk?

Reply 7: Thank you for your valuable comment. Since there were no patients with pneumothorax in this study, the association between distance to pleura, positive pressure ventilation, and pneumothorax risk could not be determined. Moreover, there were no patients who used positive pressure ventilation in this study before and after the procedure. Additionally, the distance to pleura in this study was similar to that in two previous studies (10,11). Regarding distance to pleura and positive pressure ventilation, which are risk factors for pneumothorax, we have additionally described them in the discussion section (see Page 17, lines 317-331).

In addition, as suggested by Reviewer B, more patients were added by extending the period to make the data more meaningful. By November 2022, EMN-TTNB was performed on six additional patients. Thus, we reanalyzed a total of 32 patients. All related text contents, tables, and figures were changed. Excluding four indeterminate cases, the diagnostic accuracy was 21/28 (75.0%), similar to the previous diagnosis rate of 17/22 (77.3%).

Changes in the text: The anesthesia method may affect the incidence of pneumothorax during EMN-TTNB. *Because this study was conducted under moderate sedation, no patient received positive pressure ventilation; therefore, pneumothorax is less likely to occur (19). However, moderately sedated patients move more in the lateral decubitus position, have more difficulty maintaining the position, and breathe more irregularly than deeply sedated patients. Eventually, these factors increase the risk of pneumothorax. In addition, the distance from pleura to lesion may affect the incidence of pneumothorax. The average distance from pleura to lesion in previous studies were 12.6 mm and 13.2 mm, respectively (10, 11), which were similar with 15.5 mm (median) in our study.* In previous studies, the incidence rates of pneumothorax were 17.8% and 21%, respectively (10, 11), which are comparable to the incidence of pneumothorax for CT-guided PCNB (5, 6); however, it is surprising that there was no case of pneumothorax in this study despite moderate sedation. This discrepancy can be

attributed to the lower prevalence of COPD (11.5% *vs*. 38.0%, 38.7%) and larger size of *pulmonary lesions* (*36.9* mm *vs*. 27.3 mm, 20.3 mm) in our study than in the previous studies (10, 11). *In other words, larger size of lesions and small numbers may also reflect only one complication (hemoptysis).*

Although good retrospective data with moderate sedation, however, small numbers and overall diagnostic yield is low.

1. Mean size of the lesion is 36mm which comes under the category of 'lesion' in literature. Nodules by definitions are less then 30mm. Therefore, may need to change title and use the term 'peripheral pulmonary lesions' instead of nodules.

Reply 1: Thank you for this insightful and important comment. We have changed 'pulmonary nodules' to 'pulmonary lesions' in the title and the manuscript as advised (see Page 1, line 3, Page 3, line 63, Page 4, line 73, Page 5, line 75, Page 8, lines 125,128, Page 12, line 217, Page 15, line 280, Page 17, line 329).

2. Larger size lesions and small numbers may also reflect the only one complications (Haemoptysis) and this does not match with previously reported literature.

Reply 2: We sincerely appreciate this comment and completely agree with the reviewer. Therefore, we have added your advice to the discussion (see Page 17, lines 330-331).

Changes in the text: *In other words, larger size of lesions and small numbers may also reflect only one complication (hemoptysis).*

3. Would be good to know results for lesion size smaller than 20mm.

Reply 3: Thank you for the great suggestion. We have added the data in the manuscript and Table 1 (see Page 12, lines 218-219).

	Number of patients
lesion ≤ 20 mm	6

20 < lesion ≤ 30 mm	6
lesion > 30 mm	20

Changes in the text: The mean size of the *pulmonary lesions* was *36.9±17.4* mm. *Six patients (18.8%) had lesions < 20 mm, six patients (18.8%) had lesions between 20–30 mm, and the remaining 20 patients (62.5%) had lesions > 30 mm.*

4. Is it possible to include more data on consecutive patients to make it more meaningful.

Reply 4: Thank you for this insightful comment. By November 2022, EMN-TTNB was performed on six additional patients. Thus, we reanalyzed a total of 32 patients. All related text contents, tables, and figures were changed. Excluding four indeterminate cases, the diagnostic accuracy was 21/28 (75.0%), similar to the previous diagnosis rate of 17/22 (77.3%). Of the six additional patients, four were diagnosed with cancer and were true positive and two were false negative (1. Chronic inflammation was confirmed on EMN-TTNB, but adenocarcinoma was confirmed on subsequent surgery; 2. Normal lung tissue was confirmed on EMN-TTNB; however on ENB, squamous cell carcinoma was confirmed, and the same result was confirmed on subsequent surgery). One-year follow-up is not required to confirm the diagnosis for the additional six patients. Additionally, in 32 patients, the average lesion size remained 36.9 mm. (see Page 3, lines 57, 62-66, Page 5, line 75, Page 8, line 127, Page 12, lines 212-233, Page 13, lines 234, 240-241, 243-245, 246-254, Page 15, lines 270-271, 279-280, 283, 288, Page 16, lines 292, 304, Page 17, line 329)

5. Were lesions accessible with other techniques eg navigation bronchoscopy or so & risk of pneumothorax?

Reply 5: We appreciate the reviewer's comments. In fact, when bronchial sign was positive in peripheral lung lesions, ENB was also performed along with EMN-TTNB. ENB was performed in 14 of 32 patients in this study, and lung cancer was diagnosed with ENB in two of the seven false negatives. Moreover, there were no specific

complications including pneumothorax after ENB.

6. In discussion section lines 263, 264 — author mentioned the 'overall diagnostic yield of EMN-TTNB under moderate sedation for lung cancer was 77.3%. This should be written as 'overall diagnostic yield is 77.3% excluding indeterminate cases'. Diagnostic yield for lung cancer is 10/17 58% excluding FN or for malignancy it was 10/22 including FN (45.5%). This should be mentioned in result section as well.

Reply 6: Thank you for this insightful comment. We apologize for any mistake in using diagnostic yield and diagnostic accuracy. We corrected the terminology and the content as advised in the text and Tables 2 3. (see Page 1, line 2, Page 3, lines 54, 61, 64, 69, Page 3, line 73, Page 5, line 75, Page 7, lines 118,120, Page 11, lines 198, 204, 207, Page 12, line 213, Page 13, lines 246-247, 251, Page 14, lines 263-265, Page 15, lines 269-270, 279, Page 16, line 294, Page 18, lines 354, 361)

Changes in the text:

Page 13, lines 246-247

The final diagnostic *accuracy* of EMN-TTNB was *75.0% and diagnostic yield for lung cancer is 14/21(66.7%) excluding false negatives, and 14/28 (50.0%) including false negatives.*

Page 15, lines 269-270

In the present study, the overall diagnostic yield of EMN-TTNB under moderate sedation for lung cancer was *75.0%, excluding the indeterminate cases*.

7. It would also be good to elaborate limitations of performing EMN-TTN under moderate sedation, particularly patient position and the lung areas that can be accessed with ease or difficulty under moderate sedation.

Reply 7: Thank you for this insightful and important comment. We agree with the reviewer's comment. We have revised the methods and the discussion, and we also added related references. (see Page 9, lines 160-161, Page 18, lines 344-353)

Changes in the text:

Page 9, lines 160-161

With moderate sedation, it is difficult to insert a bronchoscope for the registration process in the prone position; therefore, EMN-TTNB has to be performed in the lateral *decubitus* position for *lesions* that are difficult to access *in the supine position.* If a lateral approach was required, CT had to be performed in the same lateral *decubitus* position as that adopted during the procedure.

Page 18, lines 344-353

Second, *EMN-TTNB under moderate sedation cannot be performed in the prone position because of the registration process using bronchoscopy. When performing CT-guided PCNB or EMN-TTNB under deep sedation, usually the anterior or middle mediastinal lesions are approached from the supine position, and the middle, posterior mediastinal lesions are approached from the prone position (20). Therefore, EMN-TTNB under moderate sedation, the middle, posterior mediastinal lesions have no choice but to approach it in the lateral decubitus position. However, it is difficult to maintain posture in the lateral decubitus position and the chest wall motion is greater than in the prone position (21). Considering that the safety and success of EMN-TTNB depend on patient cooperation, more attention should be paid to sedation and posture, especially in the lateral decubitus position.*

Reviewer C

The current study investigates the outcome of ENB from peripheral nodules in 26 patients investigated in moderate conscious sedation.

1. A similar study was published from another Korean site in 2021 (https://pubmed.ncbi.nlm.nih.gov/34441366/) on 30 patients, from Turkey in 2016 on 56 patients (https://pubmed.ncbi.nlm.nih.gov/27386477/) and Japan 2018 on 35 patients (https://pubmed.ncbi.nlm.nih.gov/30034825/).

Neither of these studies are cited in the present manuscript.

Reply 1: Thank you for this comment. Our study is not about ENB, but about

electromagnetic navigation transthoracic needle biopsy (EMN-TTNB), which is a percutaneous biopsy of lung lesions using EMN technology. All three papers mentioned by the reviewer are studies on ENB. However, there are very few studies on EMN-TTNB (10-12), and the procedure has usually been performed under deep sedation in previous studies. Therefore, this study performed EMN-TTNB in moderate sedation which was novel. Previous papers related to EMN-TTNB are as follows:

10. Yarmus LB, Arias S, Feller-Kopman D, Semaan R, Wang KP, Frimpong B, et al. Electromagnetic navigation transthoracic needle aspiration for the diagnosis of pulmonary nodules: a safety and feasibility pilot study. Journal of thoracic disease. 2016;8(1):186-94.

11. Mallow C, Lee H, Oberg C, Thiboutot J, Akulian J, Burks AC, et al. Safety and diagnostic performance of pulmonologists performing electromagnetic guided percutaneous lung biopsy (SPiNperc). Respirology (Carlton, Vic). 2019;24(5):453-8.

12. Thiboutot J, Lee HJ, Silvestri GA, Chen A, Wahidi MM, Gilbert CR, et al. Study Design and Rationale: A Multicenter, Prospective Trial of Electromagnetic Bronchoscopic and Electromagnetic Transthoracic Navigational Approaches for the Biopsy of Peripheral Pulmonary Nodules (ALL IN ONE Trial). Contemporary clinical trials. 2018;71:88-95.

Comments:

2. The most widely used abbreviation of electromagnetic navigation bronchoscopy is ENB.

Diagnostic yield is calculated as "conclusive samples"/"all samples", so excluding 4/26 cases due to inconclusive results dilutes the whole meaning of diagnostic yield.

The four cases with (I guess) negative ENB-TBNB and lost to follow-up can be handled as done: as a worst-case/best-case scenario concerning NPV and PPV, sensitivity and specificity.

Reply 2: We appreciate the reviewer's comments. We apologize for any mistakes in using diagnostic yield and diagnostic accuracy. We corrected the terminology and the content as advised by Reviewer B in the text and Tables 2 and 3. Looking at the methods

(see Page 13, lines 250-254) and Table 2, the diagnostic accuracy was calculated excluding four indeterminate cases. The worst-case/best-case scenario concerning NPV and PPV, sensitivity and specificity was presented in Table 2 by assuming that the four indeterminate cases were false-negative and true-negative cases, respectively.

In addition, according to Reviewer B's suggestion, more patients were added by extending the period to make the data more meaningful. By November 2022, EMN-TTNB was performed on six additional patients. Thus, we reanalyzed a total of 32 patients. All related text contents, tables, and figures were changed. Excluding four indeterminate cases, the diagnostic accuracy was 21/28 (75.0%), similar to the previous diagnosis rate of 17/22 (77.3%).

3. Data on size and pleural distance are clearly not normally distributed as mean minus 1.96xSD provides negative numbers. For such low numbers, it is advised to use median and range.

Reply 3: Thank you for this insightful and important suggestion. We have revised our text and Table 1 as advised. For continuous variables, a normality test was performed using the Kolmogorov-Smirnov & Shapiro-Wilk test in SPSS. Age and size were considered to follow a normal distribution with a p-value greater than 0.05; however, other continuous variables, including pleural distance, did not follow a normal distribution. Thus, we changed the value as median with interquartile range. (see Page 3, lines 62-64, Page 11, line 197, Page 12, lines 216-226, Page 16, line 304-305, Table 1).

Changes in the text:

Page 3, lines 62-64:

Results: *Thirty-two* patients were enrolled (mean age *70.8±11.1* years); *56.2%* were male. The mean size of the *pulmonary lesions* was *36.9±17.4* mm, and the *median (interquartile range)* distance from the pleura was *15.5 (0.0–30.0)* mm.

Page 11, line 197

Statistical analysis

Categorical variables were described as simple proportions (%), and continuous

variables were described as means with standard deviations (SD) *or median (interquartile range, IQR).*

Page 12, lines 216-226

The mean size of the *pulmonary lesions* was *36.9±17.4* mm. *Six patients (18.8%) had lesions < 20 mm, six patients (18.8%) had lesions between 20–30 mm, and the remaining 20 patients (62.5%) had lesions > 30 mm.*

The median (IQR) distance from the pleura was *15.5 (0.0–30.0)*. The right upper and left lower lobes were the most prevalent locations at *31.2%* each, followed by the right lower lobe at *15.6%*. In all cases, the introducer needle was passed only once, and core biopsy was performed *5.0 (4.0–5.0)* times. Total duration of EMN-TTNB, defined as the time from the start of sterile field preparation prior for initial needle placement until needle removal, was *16.5 (15.0–25.3)* minutes. Midazolam and fentanyl were administered for moderate sedation at *median (IQR)* doses of *3.0 (2.0–3.0)* mg and *50.0 (25.0–50.0)* µg.

Page 16, lines 304-305

Moreover, in this study, EMN-TTNB took only *16.5 (15.0–25.3)* minutes, on *median (IQR)*, which was relatively shorter than the 18.3 minutes and 20 minutes, *on average*, respectively, in the two previous studies (10, 11).

4. The study does not add to the understanding of false-negative results.

Reply 4: We appreciate the reviewer's comments. There were seven cases of false negatives, which have already been described as follows in the text. (see Pages 12-13, lines 232-245, Pages 15-16, lines 280-294).

Page 12-13, lines 232-245

Of the *18* initially negative cases, seven *(38.9%)* were confirmed as true-negatives and *seven (38.9%)* as false-negatives. Because malignancy was clinically suspected, a different method of biopsy was performed for the *seven* patients with false-negative findings: 1) chronic inflammation was confirmed on EMN-TTNB, but squamous cell carcinoma was confirmed on subsequent surgery; 2) chronic inflammation was confirmed on EMN-TTNB, but adenocarcinoma was confirmed on subsequent surgery;

3) normal lung tissue was confirmed on EMN-TTNB, but squamous cell carcinoma was confirmed on subsequent surgery; 4) chronic inflammation was confirmed on EMN-TTNB, but adenocarcinoma was confirmed on subsequent surgery; *5) chronic inflammation was confirmed on EMN-TTNB, but adenocarcinoma was confirmed on subsequent surgery;* 6) normal lung tissue was confirmed on EMN-TTNB, but on ENB, metastatic adenocarcinoma from colorectal cancer was confirmed, and the same result was confirmed on subsequent surgery; *7) normal lung tissue was confirmed on EMN-TTNB, but on ENB, squamous cell carcinoma was confirmed, and the same result was confirmed on subsequent surgery.*

Page 15-16, lines 280-294

In this study, there were *seven* false-negative cases. In one case, the *pulmonary lesion* was difficult to access because it was located near the heart. In another case, it was relatively deep and difficult to access because it was located at the end of the outer one-third of the lung zone. In *three* out of *seven* patients, since the procedure was performed in the lateral decubitus position, it is thought that the CT-body divergence error was maximized in comparison to that in the supine position. CT-body divergence means that the CT scan before the procedure does not perfectly match the patient's airway direction at the time of the procedure due to changes in the patient's orientation (16). This eventually creates a serious difference between real lesion and virtual target. In addition, skin shift occurred in three *(9.4%)* out of *32* patients, and one of them was confirmed as a false-negative case, which is considered to be a factor that reduces the accuracy of diagnosis. Skin shift is a phenomenon in which the location of the consumable tip shown on the screen and the actual tip location are different (17). Furthermore, the fact that the *five* out of *seven* patients were examined in the initial period of the use of EMN-TTNB before the operator became accustomed to the procedure may have had some influence on the diagnostic *accuracy.*

5. In conclusion:

The studies adds little/nothing to existing evidence.

Recruitment was done in only 6 months in 2021.

If the authors chose to include >60 patients, they would provide the largest case-series, which could be of interest to ENB-operators.

Reply 5: Thank you for this comment. As we mentioned in **Reply 1**, our study is not about ENB, but about EMN-TTNB. There are very few studies on EMN-TTNB, and the procedure has usually been performed under deep sedation in previous studies. Therefore, this study, performed EMN-TTNB in moderate sedation, is valuable. In addition, the recruitment period was extended to 18 months from May 2021 to November 2022, and a total of 32 patients were reanalyzed by adding six patients.

\<Additional fixes>

There was a typographical error in Figure 1; thus, ')' was deleted as follows"

Original: ....1 year follow-up CT) (n=4)

Revised: ....1 year follow-up CT (n=4)