Peer Review File

Article Information: https://dx.doi.org/10.21037/tlcr-23-264

Reviewer A

Despite the interesting topic, I think that the paper had some important issues that do not make it suitable for publication in this version. First, the current guidelines for the mediastinal nodal staging of ESTS and ESGE recommend the use of EBUS and EUS to biopsy all the mediastinal station. Indeed, EUS had an higher sensitivity in station 7 or 5 compared to EBUS. The authors reported only the use of EBUS and the they should discuss better this.

The authors correctly reported that the oncogene addicted patients had an higher rate of nodal metastasis. Is not understood how there is a EBUS failure in these cases.

Reply: Thank you for your valuable feedback. As you mentioned, we are aware that the guidelines recommend a combined transesophageal approach. Although it is impractical for all patients in our daily clinical practice, we have aggressively combined it in cases where we believe it is necessary. We have added the following text to the "Equipment and Procedures" section to make this clear.

Changes in the text (On pages 7–8, lines 23–1): Although not performed routinely, transesophageal endoscopic ultrasound with bronchoscope-guided fine needle aspiration (EUS-B-FNA) was also aggressively conducted for cases with LNs with difficult transbronchial access, such as #5 and #8.

In addition, in response to points raised by other reviewers, we have reorganized and redescribed some of the methods and results of this study. Through this, the failure causes of nodal staging were clearly defined and redivided into the group that was punctured but failed and the group that was not punctured and failed. The latter was subdivided into the following three categories: i) difficult-to-reach LNs, ii) omission at lower level LNs due to false-positive ROSE results at the higher level, and iii) LNs with non-significant EBUS findings (i.e., <5 mm in the short diameter, not corresponding to the puncture target). As you can see in the revised Table 3, which summarizes the failed cases, the seven cases that were not punctured due to non-significant EBUS findings were all adenocarcinomas with various driver oncogenes. As argued in the Discussion, an increased incidence of subclinical LN metastasis in *EGFR* mutation-positive and *ALK* translocation-positive adenocarcinomas was reported, and LN metastases that are difficult to detect on imaging, including EBUS, may be present in cases with driver oncogenes. Therefore, the possibility that nodal staging by EBUS-TBNA may be underestimated in these cases is an important implication, and we hope you understand this point.

Reviewer B

Comment: We read with great interest the manuscript titled "Identifying factors causing failure of nodal staging by endobronchial ultrasound guided transbronchial needle aspiration in non-small cell lung cancer" submitted to your journal by So et al for publication. This is a single center, retrospective study, evaluating patients with NSCLC who underwent mediastinal staging with EBUS-TBNA. The study aims to identify factors associated with a failure of nodal staging by EBUS-TBNA. The authors identified patients with NSCLC and explored "pre-treatment" vs. "final" cancer stage in patients who did and did not undergo EBUS-TBNA staging. The authors then focus on patients who "failed" staging and identify 3 group categories: "false negative", "not approachable", and "not punctured". Finally, the authors attempted to identify clinical and oncologic factors associated with failure to stage.

After reviewing the manuscript, we would like to offer the following comments:

Reply: Thank you for your time and expertise in reviewing our work. We have prepared responses to your comments in a question-answer format. Please review them, and if you have any further questions or concerns, feel free to let us know.

Major comments:

Ethical: Authors obtained IRB approval and mentioned that informed consent requirement was waived given retrospective nature of study. They should however also mention how data was stored and de-identified to protect PHI.

Reply: Thank you for your feedback. In cases where bronchoscopic examinations have been conducted, we reference various information from electronic medical records. We assign research numbers, anonymize the data, and enter it into a database. During this process, we also maintain a separate record that links patient identifiers with these research numbers. Our database does not contain any data that could personally identify patients, and we have implemented measures to ensure that third parties cannot discern any identifying information. We have added the following text to inform readers of our commitment to protected health information.

Changes in the text (On page 6, lines 15–17): However, the information used in this study was entered into a database independent of the electronic medical record, and patient identification was controlled by a separately assigned study number so that third parties cannot identify them.

Language: although overall well-written, this manuscript is plagued by grammar inaccuracies and use of non-standardized scientific terms. It would therefore benefit from professional scientific and language editing.

Reply: Thank you for your suggestion. To address this issue, we had our manuscript professionally edited by a native English speaker before resubmission, ensuring consistent language and adherence to scientific terminology throughout the entire document.

Study population: the main concern regarding this manuscript is the heterogeneous nature of the study population examined. Th authors describe a cohort of NSCLC patients during two phases of their management: a) "pre-treatment", and b) "final". First, the group titles are confusing. Our understanding is that the "pre-treatment" group reflects clinical +/- EBUS staging, while the "final" group reflects surgical and/or MDT consensus staging. Having one group termed "pre-treatment" implies that the other group is "post-treatment", which is not necessarily the case. We strongly recommend changing the group title terms to better reflect the different phases in the patients' management.

Reply: Your feedback is valid, and we apologize for any confusion caused. Following the suggestions provided, we have made the changes from "pre-treatment" to "unconfirmed" and from "final" to "confirmed." Furthermore, we have adjusted the text to ensure that the definitions of these terms are clearly informed, as follows.

Changes in the text (On page 8, lines 4–8): Nodal stages were investigated separately at diagnosis and at treatment. The former was categorized as "unconfirmed," relying on the results of EBUS-TBNA in addition to imaging modalities such as CT and FDG-PET. In contrast, the "confirmed" nodal stages were determined based on the pathological stages of patients who underwent surgery. For patients who did not undergo surgery, the confirmed stages were based on the consensus results of the multidisciplinary team.

Second, these categories encompass 4 different groups of patients which can be illustrated by a 2x2 table as "clinical staging" with and without EBUS (2 groups) and "final staging" with patients who went for surgery vs. patients who went to MDT consensus (2 groups). These are fundamentally 4 different groups of patients in terms of clinical course, management alternatives, and clinical outcomes, including progression-free survival and overall survival. The authors consolidate all "pre-treatment" patients into a single group, including patients who did not undergo EBUS-TBNA. It is not clear from the manuscript, how decision to perform or not perform EBUS-TBNA staging was determined. It is also not clear how many patients underwent and did not underwent EBUS staging. It is the opinion of this reviewer, that since this manuscript is aimed towards identifying factors associated with failure of EBUS-TBNA staging, perhaps patients who did not undergo EBUS-TBNA should be separated from the main cohort. Similarly, the distinction between the two different "final" staging patient groups should be emphasized. The exact size of each of the above 4 groups should be clearly mentioned in the manuscript.

Reply: Following your first point of this feedback, we apologize for any confusion caused. First of all, this study included only patients who underwent nodal staging by EBUS-TBNA. The rows in the corresponding table presented the two "unconfirmed stages (the modified term)" to show how the staging shift occurred from noninvasive imaging alone (i.e., CT±FDG-PET) (A) to plus EBUS-TBNA (B). We have simplified the table, leaving only the information in (B), and moved

it to the supplementary file. On top of that, and in light of the points you indicated, we have modified the relevant results as follows.

Changes in the text (On page 10, lines 1–6): All but 11 cases were evaluated with FDG-PET in conjunction with CT. However, 189 cases (71.6%) were correctly staged without EBUS-TBNA, whereas with the addition of EBUS-TBNA, 243 cases (92.0%) were correctly staged (Table S1). There were 21 cases (7.9%) that were upstaged and 33 cases (12.5%) that were downstaged. In other words, despite the addition of EBUS-TBNA, 21 cases (8.0%) failed the nodal staging. Although we analyzed the association between the failure cases and the clinical factors, there were no significant differences (Table 2).

Definitions of EBUS failure: the authors identify 3 groups of EBUS outcomes: "false negative", "not approachable", and "not punctured". This classification is first described in the Results section, while it would be appropriate to describe it in the Methods. Further, clear definitions of the 3 groups are crucial for the understanding of this study. For example, how was "false negative" determined? What was the gold standard? Imaging? Surgery? Additionally, while the first and second groups both represent operator/technology failure, the third group represents clinical decision making at the time of the procedure. While the authors describe the comprehensive methodology of lymph node staging in their institution in the Methods, it can be assumed that patient included in this third group were "not punctured" based on clinical determinants of low yield or low risk for metastatic disease. In fact, these patients were staged by the sonographic appearance of the target lymph nodes. Therefore, this group cannot be described as EBUS failure to stage, as the decision not to puncture was clinically sound. This calls into question the analysis described in the last two paragraphs of the Results and in Table 3. We encourage the authors to revisit their definitions and adjust the analysis accordingly. It is also the opinion of this reviewer that the finding of higher rate of staging failure in patients with adenocarcinoma with oncogenic drivers may therefore represent a confounder and may lose statistical significance once the staging groups are appropriately rearranged.

Reply: You are absolutely correct, and we had described the results with unclear definitions, and we apologize first of all for that. As you indicated, we have clarified the definitions for "nodal staging" and "its success or failure and associated factors" in this study, respectively, and specified the following in the Methods.

Changes in the text (On page 8, lines 4–8): Nodal stages were investigated separately at diagnosis and at treatment. The former was categorized as "unconfirmed," relying on the results of EBUS-TBNA in addition to imaging modalities such as CT and FDG-PET. In contrast, the "confirmed" nodal stages were determined based on the pathological stages of patients who underwent surgery. For patients who did not undergo surgery, the confirmed stages were based on the consensus results of the multidisciplinary team.

Changes in the text (On page 8, lines 14–19): We defined cases where the unconfirmed and confirmed stages matched as "nodal staging successes," while those that did not match were

defined as "failures." In this study, we focused on the failure cases and explored factors associated with them. Specifically, since there is an essential difference between a) cases that were punctured but resulted in failures and b) cases that were not punctured and resulted in failures, these two groups, "punctured" and "not-punctured" were investigated separately.

Please note that the failure factors were divided into the "punctured" and "not-punctured" groups due to the essential differences as described above, and the former necessarily represents false negatives by EBUS-TBNA, while the latter must be an exploratory categorization because the causes that were not punctured are fundamental to this study that had not been examined before. We would appreciate your understanding in this regard. The corresponding results are as follows.

Changes in the text (On page 10, lines 7–12): Next, all 21 patients who failed the nodal staging are summarized (Table 3); 10 were in the punctured group and 11 were in the not-punctured group. The latter, when reviewed in detail down to the causes that prevented punctures, were subdivided into the following three categories: i) two of difficult-to-reach LNs, ii) two of omission at lower level LNs due to false-positive ROSE results at the higher level, and iii) seven of LNs with non-significant EBUS findings (i.e., <5 mm in the short diameter, not corresponding to the puncture target).

On that basis, as you can see in the revised Table 3, which summarizes the failed cases, the seven cases that were not punctured due to non-significant EBUS findings were all adenocarcinomas with various driver oncogenes. As argued in the Discussion, an increased incidence of subclinical LN metastasis in *EGFR* mutation-positive and *ALK* translocation-positive adenocarcinomas was reported, and LN metastases that are difficult to detect on imaging, including EBUS, may be present in cases with driver oncogenes. Therefore, the possibility that nodal staging by EBUS-TBNA may be underestimated in these cases is an important implication, and we hope you understand this point.

Editing and scientific comments:

EBUS-TBNA staging in clinical stage 1 peripheral disease is controversial. Please clarify your institutional approach to these cases to justify case inclusion in the manuscript. Also please change "selection" to "inclusion" in paragraph #1 of the results.

Reply: Thank you for your instruction. We have added the following text regarding our institutional standard for nodal staging by EBUS-TBNA. We have also revised from "selection" to "inclusion" as you noted.

Changes in the text (On page 6, lines 7–10): Note that we perform systematic nodal staging based on imaging, usually for cases of suspected mediastinal LN metastasis, and often also for cases of centrally located primary tumors and/or N1. We do not cover cases in which extensive mediastinal LN metastases are evident, nor do we establish criteria based on primary tumor size.

Results, paragraph #3: this is a major paragraph in the Results section and needs to be rephrased for more clear language. The confusing terms "pre-treatment" and "final" are discussed above. The use of the word "only" implies the authors' opinion and should be avoided. Although can be inferred from Table 2, some textual data about the rates of upstaged and downstaged cases would be helpful. The authors indicate that clinical factors for failure were explored, but do not elaborate which factors or provide data. This should be addressed in both Methods and Results. If word count becomes a limiting factor, consider a supplementary appendix.

Reply: We apologize again for the unclear definitions and the resulting unclear results. As answered above, we have revised the relevant sections throughout. Table 2 was just confusing, so we have simplified it and moved it to the supplementary file. On top of that, and in light of the points you indicated here, we have modified the relevant results as follows.

Changes in the text (On page 10, lines 1–6): All but 11 cases were evaluated with FDG-PET in conjunction with CT. However, 189 cases (71.6%) were correctly staged without EBUS-TBNA, whereas with the addition of EBUS-TBNA, 243 cases (92.0%) were correctly staged (Table S1). There were 21 cases (7.9%) that were upstaged and 33 cases (12.5%) that were downstaged. In other words, despite the addition of EBUS-TBNA, 21 cases (8.0%) failed the nodal staging. Although we analyzed the association between the failure cases and the clinical factors, there were no significant differences (Table 2).

Results, paragraph #4: "relatively large" is a vague term. Rephrase the statement to include core data.

Reply: Thank you for pointing this out. In accordance with the reorganization of the methods and results, we have removed the relevant part of the text in its entirety.

Paragraph #5 in the Introduction needs to be rephrased due to unclear language.

Reply: We apologize for the unclear language. We have rephrased the relevant paragraph as follows.

Changes in the text (On pages 5–6, lines 20–1): Hence, while EBUS-TBNA offers high reliability in nodal staging, it may not be infallible, prompting the need for an in-depth investigation of factors contributing to the diagnostic failures. In the quest for enhanced diagnostic precision and the ever-evolving landscape of precision medicine, this study aimed to identify factors that may contribute to nodal staging failures via EBUS-TBNA in NSCLC.

Results, Table 1: while primary lesion sampling by EBUS-TBNA may be interesting, it is not part of the staging process and cannot define successful or unsuccessful staging.

Reply: Thank you for your valuable feedback. As I responded to your point above, we perform systematic nodal staging based on imaging, usually for cases of suspected mediastinal LN metastasis, and often also for cases of centrally located primary tumor and/or N1. We also routinely

perform ROSE, and based on the results, repeat the puncture in the order of N3, N2, N1 stations, and primary lesion until adequate staging is confirmed. Therefore, the centrally located primary tumor is one of the conditions subject to nodal staging, and if ROSE does not confirm N1 or higher, it would be considered puncturing the primary lesion, if approachable. Thus, please understand that primary lesions are not necessarily irrelevant to the staging process, which we consider an important part of it.

Results, Table 1 and paragraph #2: please indicate how often and which lesions were preferentially sampled by EUS-B-FNA.

Reply: Thank you for your insightful suggestions. We have modified Table 1 to show the site and number of lesions sampled by EUS-B-FNA. To avoid any complications, we have not made any changes to the text.

It would be interesting to know the mean or median time between the clinical staging (PET-CT/CT), pathologic staging with EBUS and surgical staging, as long intervals between them will limit comparison.

Reply: Thank you for your valuable feedback. All but seven patients underwent EBUS-TBNA within one month after CT/FDG-PET, and in seven cases, EBUS-TBNA was performed within a maximum of two months. After diagnosis, treatment was promptly initiated. However, the speed of treatment varied depending on the department and the physician in charge of the patient at the time of the initial diagnosis. Even if specific figures were provided regarding the treatment duration, the impact would be far from scientific; therefore, we chose not to include them in the text.

Methods, section 2.1: "extracted"=captured? Please rephrase to avoid confusion with "excluded". Also- please elaborate on the inclusion criteria. In he Results, it would be useful to know the distribution of NSCLC cases among your cohort.

Reply: We apologize for the confusion caused; it has been corrected. We have modified the inclusion and exclusion criteria for clarity as follows. As answered above, our institutional standard for nodal staging by EBUS-TBNA has also been added here.

Changes in the text (On page 6, lines 5–12): Consecutive patients who underwent EBUS-TBNA at our hospital between January 2017 and December 2020 were reviewed. Of them, cases wherein the purpose was systematic nodal staging were included. Note that we perform systematic nodal staging based on imaging, usually for cases of suspected mediastinal LN metastasis, and often also for cases of centrally located primary tumors and/or N1. We do not cover cases in which extensive mediastinal LN metastases are evident, nor do we establish criteria based on primary tumor size. Cases for i) re-staging purposes after any treatment, and ii) those found to be stage IV thereafter were excluded. Finally, iii) cases diagnosed with other than NSCLC were also excluded, and the remaining cases were eligible for analyses.

Reply: In addition, we have provided more details on the histological type of distribution in Table 1 and also described in the Results as follows.

Changes in the text (On page 9, lines 22–24): The histologic type was mostly adenocarcinoma in 154 (58.3%), followed by squamous cell carcinoma in 77 (29.2%), NSCLC, NOS in 13 (4.9%), and others in 20 (7.6%).

Methods, section 2.2: "to make the patient more comfortable" – this is an unnecessary statement. Pthidine is a brand name. Please use generic medication names. Please define how bronchoscopy experts were defined.

Reply: Thank you for pointing this out. We have removed the "to make the patient more comfortable" statement. It appears that "pethidine" also has another name, "meperidine," but since both are recognized as generic names, we have not changed this description. On the other hand, we have added to the definition of bronchoscopy experts "with more than 10 years of experience."

Methods, section 2.2: 3rd line please correct the term "those". Please describe the procedure in a scientific manner. The use of the terms "usually" and "up to 30-50" is not scientifically sound and implies non-standardization. Please also indicate how often ROSE was employed.

Reply: Thank you for your instructions. We have modified it as follows.

Changes in the text (On pages 7–8, lines 16–1): Subsequently, targeted lesions with sonographic findings suggestive of malignancy (22, 25) or at locations critical to determine the treatment protocols were punctured in the order of the N3, N2, N1 stations, and primary lesion (if approachable) to avoid contamination from higher LNs. EBUS-TBNA was performed using a negative pressure syringe (10 or 20 mL) with around 30–50 agitations per puncture. If there was considerable blood backflow, lower negative pressure or a slow-pull method was applied to the subsequent passes. Whenever possible, the puncture was repeated at least twice to ensure the sample adequacy, while referring to the results of rapid on-site cytologic evaluation (ROSE). Although not done routinely, transesophageal endoscopic ultrasound with bronchoscope-guided fine needle aspiration (EUS-B-FNA) was also aggressively performed for cases with LNs with difficult transbronchial access, such as #5 and #8.

Methods, section 2.4: this methodology is flawed, as indicated above. Please rephrase and consider reorganizing.

Reply: Thank you again for pointing this out. As answered above, according to your indications, we have clarified the definitions for "nodal staging" and "its success or failure and associated factors" in this study, respectively, and specified the following in the "Definition of nodal stage" subsection.

Changes in the text (On page 8, lines 4–8): Nodal stages were investigated separately at diagnosis and at treatment. The former was categorized as "unconfirmed," relying on the results of EBUS-

TBNA in addition to imaging modalities such as CT and FDG-PET. In contrast, the "confirmed" nodal stages were determined based on the pathological stages of patients who underwent surgery. For patients who did not undergo surgery, the confirmed stages were based on the consensus results of the multidisciplinary team.

Conclusions: please rephrase. The Conclusion should not be "copy/pasted" from the prior segments of the manuscript, but rather summarize your main findings and conclusions.

Reply: Thank you very much for your valid points. We have summarized and revised the conclusion as follows.

Changes in the text (On page 14, lines 17–22): The present study demonstrated the risk of false positives with ROSE and the involvement of driver oncogenes as factors associated with nodal staging failure in NSCLC by EBUS-TBNA, in addition to limitations of the procedure itself, including sampling performance and reachability. Especially in adenocarcinoma patients with driver oncogenes, the possibility of metastases was significantly high even in LNs with obscure image findings, and their nodal staging results should be interpreted cautiously.

Tables 1+3 include non-standardized lymph node stations. Do #11s and #11i refer to the left or the right hilum? Similarly: 12u, 12l, 13, 14?

Reply: Thank you for pointing this out. #11s: between the upper lobe bronchus and bronchus intermedius on the right and #11i: between the middle and lower lobe bronchi on the right, are clearly stated in the IASLC map and seem to have been standardized (DOI: 10.1097/JTO.0b013e3181a0d82e). These originated from the Japan Lung Cancer Society Map, and as you pointed out, the notation of #12u/m/l is not standardized in IASLC. Therefore, we have changed the notations to R/L throughout to make them consistent.

Table 2: terms used to describe Columns (A) and (B) are confusing. Suggested terminology would be; (A) – Clinical staging alone, (B) EBUS staging alone.

Reply: Thank you for pointing out a valid point. Table 2 is just confusing as you said. We decided to list only the clinical nodal stage (B) with imaging (CT, FDG/PET) plus EBUS-TBNA and moved this to the supplementary table.

Table 3- the "punctured lesion" column includes lesions that were not punctured due to inaccessibility, which is confusing. Similarly, while for some cases lesions not punctured due to accessibility are documented in the "punctured lesion" column in parentheses, for other cases they are not. Please revise the table for more clarity and perhaps differentiate cases based on the reasons the lesions were not sampled or failed sampling.

Reply: Your point is valid, and we apologize for any confusion. We have revised Table 3 to reflect the new classification of failure causes, and the remaining information has been rewritten.

Page 3, Line 13-14. Reference (1) should be moved to the end of sentence.

Reply: Thank you for pointing out the impropriety, it has been corrected.

Changes in the text (On page 4, lines 14–16): Lung cancer is the leading cause of cancer-related deaths, with an estimated 2.2 million new cases and 1.8 million deaths worldwide in 2020, among which 85% of the deaths were due to non-small cell lung cancer (NSCLC) (1).

Page 3, Line 15, to read Accurate nodal staging is crucial for the prognosis and treatment of NSCLC (reference)

Reply: Thank you for pointing out the mistake, it has been corrected.

Changes in the text (On page 4, lines 17–18): Accurate nodal staging is crucial for the prognosis and treatment of NSCLC (2–5) without distant metastases.

Page 3, Line 22: change to …for sampling of.

Page 3, Line 23: de-abbreviate LNs

Reply: Thank you for your suggestion. We have revised the sentence as follows.

Changes in the text (On pages 4–5, lines 24–2): Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive and standardized technique for sampling of lesions contacting the central airway, especially hilar and mediastinal LNs.

Page 3, Line 25: Rephrase the sentence. Replace terms like cheaper with cost effective.

Reply: Thank you for your suggestion. We have revised the sentence as follows.

Changes in the text (On page 5, lines 3–4): Thus, when utilized as the initial examination, it is safer, more cost-effective, and equally as efficient as surgical nodal staging (11–17).

Page 3, Line 33-36: reference needed

Reply: With the addition of references and in light of the latest findings, we have revised the sentence as follows.

Changes in the text (On page 5, lines 10–13): While current guidelines recommend that cases of negative EBUS-TBNA with strong suspicion of LN metastases should undergo additional surgical staging, such as cervical mediastinoscopy (8, 19), a recent randomized controlled trial has reported the validity of omitting it (20).

Page 3, Line 37: there is a lot of evidence that establishes the accuracy of EBUS TBNA. Thus statement is partly incorrect.

Reply: Thank you for your valuable feedback. To inform the significance of this study regarding the detailed investigation of failure factors of nodal staging, we have rephrased the relevant paragraph as follows.

Changes in the text (On page 5, lines 20–24): Hence, while EBUS-TBNA offers high reliability in nodal staging, it may not be infallible, prompting the need for an in-depth investigation of factors contributing to the diagnostic failures. In the quest for enhanced diagnostic precision and the ever-evolving landscape of precision medicine, this study aimed to identify factors that may contribute to nodal staging failures via EBUS-TBNA in NSCLC.

Page 4, Line 43: Rephrase the sentence: Each bronchoscopist.

Reply: Thank you for your valuable feedback. We have connected that sentence with the previous one and rephrased it as follows.

Changes in the text (On page 7, lines 21–23): Whenever possible, the puncture was repeated at least twice to ensure the sample adequacy, while referring to the results of rapid on-site cytologic evaluation (ROSE).

Page 5, Line 2: Remove "Moreover, sometimes".

Rephrase suggestion; …Transesophageal…was used for lymph nodes with difficult transbronchial access

Reply: Thank you for your appropriate remark. Following your suggestion, we have rephrased that sentence as follows.

Changes in the text (On pages 7–8, lines 23–1): Although not performed routinely, transesophageal endoscopic ultrasound with bronchoscope-guided fine needle aspiration (EUS-B-FNA) was also aggressively conducted for cases with LNs with difficult transbronchial access, such as #5 and #8.

Page 6, Line 7: change "in" to "on"

Reply: Thank you for pointing out the impropriety, it has been corrected.

Changes in the text (On page 9, lines 20): The primary lesions were located mainly on the right side (175 cases [66.3%]) and at the peripheral location (209 cases [79.2%]).

Page 6, Line 44: change "failures" to "failure"

Reply: Thank you for pointing out the impropriety, it has been corrected.

Changes in the text (On page 11, lines 6): To the best of our knowledge, few studies have investigated the factors leading to the failure of nodal staging by EBUS-TBNA and evaluated their relationship to driver oncogenes.

Page 8, Line 28 -30: statement is not accurate, recommend removal.

Reply: Thank you for your valuable feedback. We have removed the relevant section. As you pointed out above, we have summarized and revised the conclusions as follows.

Changes in the text (On page 14, lines 17–22): The present study demonstrated the risk of false positives with ROSE and the involvement of driver oncogenes as factors associated with nodal staging failure in NSCLC by EBUS-TBNA, in addition to limitations of the procedure itself, including sampling performance and reachability. Especially in adenocarcinoma patients with driver oncogenes, the possibility of metastases was significantly high even in LNs with obscure image findings, and their nodal staging results should be interpreted cautiously.

Reviewer C

Passion for the topic and a lot of work emerge but, unfortunately, the methodology is inappropriate and there are many papers in the Literature which, in a more systematic and precise way, describe the limits of the procedure (doi: 10.1186/s12931-023-02321-9.)

For more details, see the attached file.

Page 3, Lines 18–20: Unclear sentence.

Reply: We apologize for the unclear sentence. We have revised the sentence as follows.

Changes in the text (On page 4, lines 19–23): Therefore, invasive nodal staging involving the collection of pathologic specimens from lymph nodes (LNs) is recommended for patients suspicious of mediastinal LN metastasis (N2 or higher) and is also considered in the presence of the following: primary lesion >3 cm, primary lesion centrally located, or N1 suspected on imaging (6–8).

Page 4, Lines 3–7: Please, better describe the number of different groups.

Reply: We apologize for the unclear description. We have numbered and clarified the exclusion criteria as follows. Please note that we have also added our usual nodal staging coverage at our hospital, following instructions from another reviewer.

Changes in the text (On page 6, lines 5–12): Consecutive patients who underwent EBUS-TBNA at our hospital between January 2017 and December 2020 were reviewed. Of them, cases wherein the purpose was systematic nodal staging were included. Note that we perform systematic nodal staging based on imaging, usually for cases of suspected mediastinal LN metastasis, and often also for cases of centrally located primary tumors and/or N1. We do not cover cases in which extensive mediastinal LN metastases are evident, nor do we establish criteria based on primary tumor size. Cases for i) re-staging purposes after any treatment, and ii) those found to be stage IV thereafter were excluded. Finally, iii) cases diagnosed with other than NSCLC were also excluded, and the remaining cases were eligible for analyses.

Page 4, Lines 28–29: Inappropriate sentence. Limited pre-procedural imaging due to insurance coverage and radiologist skill are the main factors.

Reply: Thank you for your comment. Unfortunately, I do not understand the intent of your comment. This subsection is about our equipment and procedures and has nothing to do with insurance coverage and/or radiologist skills. Therefore, please understand that we have not made any changes to the relevant sentence.

Page 5, Lines 28–29: Please, the describe the aim for this classification.

Reply: We apologize for the inadequate description. Among N2-positive cases, the difference between single and multiple stations has a significant impact on the indication for surgery, and we have added this point as follows. Although not yet publicly available, this difference will be reflected in the next edition of the TNM classification in discussions at the IASLC Staging Committee.

Changes in the text (On page 8, lines 9–11): Since the prognoses have been reported to differ between patients with single- and multiple-station N2 (N2a and N2b, respectively) (26), and the difference has a significant impact on the indication for surgery, they were investigated separately.

Page 6, Lines 2–4: Unclear sentence.

Reply: Thank you for your comment. As indicated in the response above, we have added our usual nodal staging coverage at our hospital as well as numbered and clarified the exclusion criteria. The number of patients to be analyzed is now clear when combined with the flow diagram in Figure 1, so please check it again.

Page 6, Lines 11–18: Please, better define. How you can stage without EBUS-TBNA?

Reply: We appreciate your insightful inquiry. We had contrasted nodal stage based on imaging alone with nodal stage based on imaging plus EBUS-TBNA, and the transition was shown in Table 2. However, as other reviewers pointed out, this was only confusing. Therefore, we have focused on the nodal stage in which EBUS-TBNA was added to the imaging findings and moved the table to the supplementary file. In the main text, only a summary showing the validity of staging by EBUS-TBNA was left.

Changes in the text (On page 10, lines 1–6): All but 11 cases were evaluated with FDG-PET in conjunction with CT. However, 189 cases (71.6%) were correctly staged without EBUS-TBNA, whereas with the addition of EBUS-TBNA, 243 cases (92.0%) were correctly staged (Table S1). There were 21 cases (7.9%) that were upstaged and 33 cases (12.5%) that were downstaged. In other words, despite the addition of EBUS-TBNA, 21 cases (8.0%) failed the nodal staging. Although we analyzed the association between the failure cases and the clinical factors, there were no significant differences (Table 2).

Page 6, Lines 19–23: Please, better define how 'failed' means.

Failed for acquisition sampling technique or due to inadequacy of the acquired cytological sample?

Reply: Thank you for pointing out the vague expressions. Other reviewers have similarly pointed out that the definition of nodal staging failure is confusing. We clarified the definition in this study and specified the following in the Methods.

Changes in the text (On page 8, lines 4–8): Nodal stages were investigated separately at diagnosis and at treatment. The former was categorized as "unconfirmed," relying on the results of EBUS-TBNA in addition to imaging modalities such as CT and FDG-PET. In contrast, the "confirmed" nodal stages were determined based on the pathological stages of patients who underwent surgery. For patients who did not undergo surgery, the confirmed stages were based on the consensus results of the multidisciplinary team.

Changes in the text (On page 8, lines 14–19): We defined cases where the unconfirmed and confirmed stages matched as "nodal staging successes," while those that did not match were defined as "failures." In this study, we focused on the failure cases and explored factors associated with them. Specifically, since there is an essential difference between a) cases that were punctured but resulted in failures and b) cases that were not punctured and resulted in failures, these two groups, "punctured" and "not-punctured" were investigated separately.

Page 6, Lines 23–28: Very inappropriate sentence. Driver mutations are more frequent in female non-smokers. Neoplastic molecular profiling cannot be associated with 'failed' sampling.

Reply: Thank you for your comment. We only described the results obtained. Furthermore, please understand that we deleted relevant sentences in the process of editing.

Page 6, Lines 33–35: As previosuly stated at lines 19-28

Reply: Thank you for your comment. The relevant part is an invariant result in the population we analyzed. I am sure you understand that we can interpret the results differently, but we cannot bend the results.

Page 6, Lines 39–44: Please, compare with 'Optimizing molecular testing of lung cancer needle biopsy specimens: potential solutions from an interdisciplinary qualitative study'. by Florian J. Fintelmann et al.

Reply: Thank you for the introduction of the paper by Fintelmann et al., which offers valuable insights into optimizing molecular testing for lung biopsy specimens in patients with advanced NSCLC. Our study primarily investigates factors causing nodal staging failure by EBUS-TBNA in NSCLC patients in order to enhance diagnostic accuracy, and as such, our research differs substantially from the study. While we cannot reference this paper as it is not directly related to our research, we appreciate the reviewer's contribution to the discussion of relevant literature.