Peer Review File

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Reviewer A

1. The author should state how to perform EBUS-TBNA whether follow ACCP guideline or follow their own protocol. The number of passes, needle size, ROSE vs non-ROSE, etc. This will reflect the diagnosis overall.

Reply: We appreciate the reviewer's constructive suggestion.

In our study, EBUS-TBNA was performed according to the standard protocol of our institution, which is largely consistent with the recommendation available at the time of data collection. We have added these details in the Methods section.

Changes in the text:

All procedures were performed according to the standard protocol of our institution, which is largely consistent with the recommendation, under local anesthesia and conscious sedation, using a convex probe EBUS bronchoscope (BF-UC180F; Olympus, Tokyo, Japan), and an ultrasound processor (EU-ME2; Olympus, Tokyo, Japan), with a 10 MHz ultrasound setting. A 21- or 22-gauge TBNA needle was employed, as previous demonstrated no significant difference in diagnostic yield between the two needle sizes. (Methods: see Page 8, line 141-146)

Transbronchial needle aspiration (TBNA) was performed on all accessible lymph nodes. For each targeted lymph node, a minimum of three passes were obtained to optimize diagnostic yield. All specimens were submitted to cytological, histopathological, and microbiological examinations after the procedure without rapid on-site evaluation (ROSE). (Methods: see Page 9, line 174-177)

2. The author should state how to capture the image and how this process standardize between patient/bronchoscopist. And who interpret image which will reflect outcome.

Reply: All procedures were continuously video recorded and subsequently reviewed by the performing operator under blinded conditions to clinical information and final diagnoses, ensuring standardized assessment across all cases.

Changes in the text:

The entire procedure was continuously video recorded directly from the ultrasound processor for documentation. (Methods: see Page 8, line 149-150)

All procedures were continuously video recorded and subsequently reviewed by the performing operator under blinded conditions with respect to clinical information and final diagnoses, ensuring that all cases were assessed in an identical manner and minimizing recall and information bias. (Methods: see Page 10, line 188-190)

3. It would be better for clinical purpose to find the factor that predict malignancy in LN

between 1-2 cm. Because this is common LN size findings in practice and need to differentiate between benign and malignant. Especially in TB endemic area. The author's data showed equal number of LN between malignant and benign.

Reply: We have added the relevant information in Table 5 and further addressed this point and mention in discussion section

Changes in the text: These challenges are particularly evident in very small lymph nodes (<10 mm), where both conventional mode and elastography may provide limited diagnostic information. By contrast, in lymph nodes of 10–20 mm, a size frequently encountered in clinical practice, predictive EBUS features for malignancy were consistent with those of larger nodes (>20 mm), suggesting their applicability across different size ranges. *(Table 5)* (Discussion, see Page 16, line 314-319)

Reviewer B

This is a retrospective study evaluating the diagnostic accuracy of an integrated approach combining conventional mode and elastography. The authors demonstrated that the combination of conventional mode and elastography provides higher diagnostic accuracy in the differential diagnosis of malignant and benign hilar and mediastinal lymph nodes. This paper is well-written and provides valuable insights to readers. I have a few comments.

Major:

1. Several features of conventional mode and elastography have been reported as predictive factors for distinguishing malignant lymph nodes from benign lymph nodes. These are important basic knowledge. I agree with the result that "the combination of conventional mode and elastography improves diagnostic accuracy for malignant lymph nodes compared to each modality alone," but I question whether it is more useful than each modality alone in clinical practice. Even if the combination is more accurate than each modality alone, it cannot replace histopathological confirmation. How will this result impact our clinical practice?

Reply: We fully agree with the reviewer's important point and have clarified this aspect in the revised version.

Although the integrative use of conventional mode and elastography improves diagnostic accuracy compared to each modality alone, it cannot replace histopathological confirmation.

Changes in the text:

Taken together, our findings highlight the potential role of integrative EBUS features as complementary tools in clinical practice. In the diagnosis and staging of lung cancer, suspicious lymph nodes require sampling regardless of EBUS features, to avoid missed diagnoses. By contrast, in benign conditions such as tuberculosis in endemic regions, EBUS features suggestive of benignity may help reduce unnecessary punctures. Similarly, in suspected extrathoracic metastases with multiple lymph node stations, EBUS features may help optimize target selection, allowing sampling to be directed toward the most suspicious nodes instead of all stations. (Discussion: see Page 17, line 330-336)

2. In multivariate analysis, non-type I patterns (types II and III) were not significant predictors of malignant tumors. Is the non-type I pattern not important in predicting malignant tumors using a combination of conventional mode and elastography?

Reply: Although non-type I patterns (types II and III) were not statistically significant predictors in the multivariable analysis, they cannot be disregarded in clinical practice. Type III patterns were observed predominantly in malignant lymph nodes, while type II patterns are inconclusive and may still harbor malignancy. Therefore, omitting tissue sampling in these groups could lead to missed diagnoses.

Changes in the text:

Classified as non-Type I, the grouping of Type II (part-blue/part non-blue, inconclusive) and

Type III (predominantly blue, highly suggestive of malignancy) was associated with malignant nodes in univariable analysis but not in multivariable analysis. Nevertheless, tissue confirmation remains essential in clinical practice, given the inherent limitations of the color classification system, to avoid misdiagnosing malignancy. False positives may occur in benign node with calcification and fibrotic component, whereas false negatives may arise in malignant nodes with necrosis or hypervascularity. (Discussion: see Page 15, line 301-307)

Reviewer C

We greatly appreciate the reviewer's constructive advice, which has guided us in refining this work and will be valuable for future research. We have made additional revisions to improve the manuscript in accordance with the reviewer's kind suggestion.

Strengths:

1. Clear Objective: The study has a well-defined objective to evaluate the diagnostic accuracy of combining conventional mode and elastography in EBUS-TBNA for malignant intrathoracic lymphadenopathy.

Reply: We are grateful for the reviewer's recognition of the clarity of our study objective.

2. Comprehensive Methodology: The methods section is detailed, providing sufficient information for replication, including participant selection, EBUS procedure, and statistical analysis.

Reply: We appreciate the reviewer's recognition of this point.

3. Significant Findings: The study demonstrates the added diagnostic value of combining conventional mode and elastography, with an AUC of 0.90, which is higher than either modality alone.

Reply: This study aimed to demonstrate the benefit of combining conventional features with elastography to improve diagnostic accuracy and reduce the limitations associated with operator dependency.

4. Transparency: Ethical approval and conflicts of interest are clearly stated, and the STARD checklist is followed for reporting diagnostic accuracy studies.

Reply: Ethical approval is stated in methods. (see Page 8, line 137-139) This manuscript was prepared in accordance with the STARD checklist. (see Page 7, line 126)

Weaknesses:

5. Single-Center Design: The study is limited to a single center and single operator, which may affect generalizability. Consider recommending multicenter studies for broader applicability.

Reply: We agree with the reviewer's comment. This limitation has been acknowledged in the Discussion, where we have also highlighted the importance of future multicenter investigations to validate and extend our findings.

6. Retrospective Nature: The retrospective design may introduce selection bias. Prospective studies could provide stronger evidence.

Reply: The retrospective design and its potential for recall and information bias have been acknowledged in the Discussion, and the need for future prospective studies has been emphasized.

7. Limited Sample Size: While 210 lymph nodes were analyzed, certain features (e.g., BA

inflow sign) were observed in a small number of nodes, which may limit statistical power.
Reply: In our study, the BA inflow sign was observed in only a small number of nodes, which may have resulted in underestimation and limited statistical power. This limitation has been addressed in the Discussion.

8. Operator Dependency: Conventional mode features are qualitative and subject to observer variability. Suggest including interobserver variability analysis in future studies.
Reply: We have added a statement in the Discussion regarding operator dependency and noted that interobserver variability analysis should be addressed in future studies, as recommended.

9. Missing Data: Some lymph nodes were excluded due to unavailable video recordings or non-diagnostic results. This could impact the robustness of the findings.
Reply: In our study, 7% of the data were missing; as they were considered missing completely at random, and managed using complete case analysis.

Suggestions for Improvement:
10. Expand Discussion: The discussion could further elaborate on the clinical implications of the findings, especially how the combined approach could be integrated into routine practice.
Reply: Additional details have been provided in the revised manuscript, in line with the reviewer's kind recommendation.

Changes in the text: In the present study, we therefore aimed to integrate fewer parameters that are simple, reliable, and suitable for real-time evaluation during the EBUS-TBNA procedure. A combined model incorporating quantitative, elastographic strain ratio > 12.48 with qualitative conventional EBUS features including absence of CHS, round shape, presence of coagulation necrosis and lymph node size > 20 mm enhanced the diagnostic accuracy for predicting malignant lymph nodes. Notably, comparable accuracy was achieved even when the strain ratio was combined with only a single conventional feature.
Taken together, our findings highlight the potential role of integrative EBUS features as complementary tools in clinical practice. In the diagnosis and staging of lung cancer, suspicious lymph nodes require sampling regardless of EBUS features, to avoid missed diagnoses. By contrast, in benign conditions such as tuberculosis in endemic regions, EBUS features suggestive of benignity may help reduce unnecessary punctures. Similarly, in suspected extrathoracic metastases with multiple lymph node stations, EBUS features may help optimize target selection, allowing sampling to be directed toward the most suspicious nodes instead of all stations. (Discussion: see Page 16, line 324-336)

11. Address Limitations: While limitations are mentioned, the manuscript could provide more detail on how these limitations might affect the results and conclusions.
Reply: As this was a single-center study performed by a single operator, the assessments, which are operator-dependent, were prone to recall and information bias. To minimize this risk, a standardized protocol for reviewing EBUS features was implemented, with patient identifiers and final diagnoses blinded during video review.

Changes in the text: There are several notable limitations. First, its retrospective design limited the completeness of video recording. Second, being a single-center, single-operator study may limit generalizability and introduce operator-dependent, recall and information bias. To minimize these potential biases, patient identifiers, clinical data, and final diagnoses were blinded. All video recordings were reviewed in a sequential manner, beginning with conventional mode and followed by elastography, to ensure consistency across cases and to minimize information bias. Third, for nodes classified as indefinite benign (absence of malignancy), pathological confirmation could not be obtained through other modalities; therefore, only clinical and radiologic follow-up was conducted. Fourth, this study has a temporal limitation, as the dataset was collected earlier and reanalyzed later. Nevertheless, the findings remain clinically relevant and may help optimize sampling strategies, minimize unnecessary procedures, and support the selective use of alternative modalities such as EBUS-guided transbronchial mediastinal cryobiopsy or intranodal forceps biopsy, particularly in cases where sarcoidosis or malignant lymphoma is suspected. (Discussion, see Page 17, line 337-348)

12. Visual Representation: Figures and tables are helpful, but consider adding a visual summary (e.g., flowchart or infographic) to highlight key findings and diagnostic performance comparisons.
Reply: We appreciate the reviewer's suggestion. In response, we have added a new table (Table 5) and an additional figure (Figure 6) to provide clearer information.

13. Future Directions: Recommend emphasizing the need for prospective, multicenter studies with larger sample sizes to validate findings and assess interobserver variability.
Reply: We have addressed the future directions in the revised Discussion section.

Changes in the text: Future studies with multiple operators, multicenter and prospective designs, a larger study population and interobserver variability assessment are warranted to better reflect clinical practice and improve generalizability. (see Page 17, line 348-351)

14. Language Refinement: Some sections could benefit from clearer phrasing to improve readability, especially in the results and discussion sections.
Reply: We are grateful for the reviewer's suggestion. We have made careful efforts to refine the language in the Results and Discussion sections to improve readability.

Specific Comments:

15. Abstract: The abstract is concise but could include more details on the clinical significance of the findings.

Reply: We have revised the Abstract to include more details on the clinical significance of the findings.

Changes in the text: The integrative use of elastography with conventional mode significantly improved diagnostic performance for distinguishing malignant lymph nodes, achieving an AUC of 0.90 (95% CI: 0.85–0.94), compared with 0.83 (95% CI: 0.78–0.89; P=0.0023) for conventional mode alone and 0.79 (95% CI: 0.73–0.85; P<0.001) for elastography alone. Combining strain ratio > 12.48 with any single conventional feature (lymph node size >20 mm, coagulation necrosis, round shape, or absence of CHS) yielded comparably high diagnostic accuracy (AUC 0.81–0.84), with no significant differences among the combinations. (Abstract, see Page 3, line 47-53)

16. Introduction: The rationale is well-stated, but the knowledge gap could be more explicitly highlighted.
Reply: Additional details have been incorporated into the Rational and knowledge gap section in response to the reviewer's suggestion.

Changes in the text: However, the part-blue/part non-blue pattern remains inconclusive, posing limitations in interpretation. Quantitative parameters including strain ratio (SR), stiff area ratio, blue color proportion, blue pixel ratio, and mean strain histogram have been utilized to enhance diagnostic reliability. However, several of these parameters require, specialized image-analysis software, potentially limiting their use in routine clinical practice.
The strain ratio is calculated by comparing the strain of a reference area surrounding the lymph node with that of the lymph node, with values directly obtained from the ultrasound processor. However, SR is subject to considerable variability and has shown lower accuracy than the color-classification method. Published studies report a wide range of cut-off values 2.47-32.07, indicating the need for further work to establish consistent thresholds for clinical application. (11-14) (Rationale and knowledge gap, see Page 7, line 98-106)

17. Methods: The strain ratio cut-off determination is well-explained, but variability across studies could be discussed further.
Reply: An expanded discussion on the variability of strain ratio cut-off values across studies has been added to the Discussion section.

Changes in the text: In our previous study, a strain ratio cut-off >2.5 provided a sensitivity of 100% for predicting malignant nodes, whereas the present study demonstrated a higher optimal strain ratio cut-off value of 12.48 with a comparable high sensitivity of 92.7%. However, strain ratio remains a parameter with substantial variability and a lack of standardized cut-off thresholds. Potential limitations include the influence of underlying etiologies, lymph node locations adjacent to vascular structures or affected by respiratory movement, inconsistency in region-of-interest selection, and operator-dependent interpretation. (Discussion, see Page 16, line 294-299)

18. Results: The diagnostic performance of individual features is well-presented, but the clinical utility of combining features could be emphasized more.
Reply: The Results has been revised to include elaboration on the clinical utility of our findings, as suggested.

Changes in the text: Moreover, the integration of SR > 12.48 with any single conventional feature—lymph node size >20 mm, coagulation necrosis, round shape, or absence of CHS—yielded consistently high and comparable diagnostic accuracy (AUC 0.84, 0.84, 0.83, and 0.81), with no significant differences among combinations (p = 0.409) *(Figure 5B)* (Result, see Page 14, line 249-252)

19. Discussion: The comparison with previous studies is helpful, but the manuscript could better address discrepancies in findings (e.g., vascular grading and BA inflow sign).
Reply: Additional explanation regarding the discrepancies observed in vascular grading and the BA inflow sign has been incorporated into the Discussion section.

Changes in the text: The BA inflow sign is commonly seen in malignant lymph nodes and associated with abundant vascularity, indicating active neovascularization. In this study, the presence of BA inflow sign showed high specificity of 96.5% and PPV of 82.4% but limited sensitivity, indicating its usefulness in confirming malignant lymph nodes. However, BA inflow sign is observed in only 17 nodes, which may reflect underestimation. This may be explained, in part, by the relatively low proportion of lymph nodes with abundant vascularity (50.5%) and by the presence of coagulation necrosis in a considerable number of nodes (54.8%), which may have limited visualization on Doppler imaging. (Discussion, see Page 15, line 227-283)

Additional Questions:
20. Timing of Submission: Why has the study been submitted at this time, despite being completed in December 2018? Clarifying the delay in submission would provide context and help assess the relevance of the findings in the current clinical landscape.
Reply: We respectfully clarify that part of the dataset was obtained retrospectively from a previous study conducted during that period. Although data collection was completed in 2018, and was subsequently interrupted during the COVID-19 pandemic. The dataset was reanalyzed and the manuscript prepared at a later stage and we believe the findings continue to provide clinical value in the present landscape. This is particularly important in settings with benign diseases such as endemic tuberculosis, where these findings may help reduce unnecessary sampling. In contrast, in cases where sarcoidosis or malignant lymphoma is suspected, these findings may support the decision to employ alternative pathological diagnostic modalities, such as transbronchial cryonodal biopsy, to improve diagnostic yield.
Changes in the text: Fourth, this study has a temporal limitation, as the dataset was collected earlier and reanalyzed later. Nevertheless, the findings remain clinically relevant and may help

optimize sampling strategies, minimize unnecessary procedures, and support the selective use of alternative modalities such as EBUS-guided transbronchial mediastinal cryobiopsy or intranodal forceps biopsy, particularly in cases where sarcoidosis or malignant lymphoma is suspected.. (Discussion, see Page 17, line 329-333)

21. Needle Type and Size: The manuscript mentions the use of 21- or 22-gauge TBNA needles but does not elaborate on the rationale for choosing these sizes or their impact on diagnostic performance. Including a discussion on this aspect would enhance the methodological clarity.
Reply: We have added these details in the Methods section.

Changes in the text: A 21- or 22-gauge TBNA needle (NA-201SX-4201/NA-201SX-4022; Olympus) was employed, as previous evidence demonstrated no significant difference in diagnostic yield between the two needle sizes. (Methods: see Page 8, line 129-131)

Conclusion:
The manuscript provides valuable insights into the integrative use of conventional mode and elastography in EBUS-TBNA. While the findings are promising, addressing the limitations, expanding on clinical implications, clarifying the timing of submission, and commenting on the choice of needle type and size would strengthen the study's impact.

Reply: We greatly appreciate the reviewer’s thoughtful feedback. In response, we have acknowledged the study’s limitations, elaborated on its clinical implications, clarified the timing of submission, and included additional discussion on the rationale for needle type and size. These revisions have been incorporated to enhance the clarity and impact of the manuscript.