Peer Review File

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Reviewer A

The authors evaluated to determine whether PTNB increases the risk of pleural recurrence in patients with clinical stage I NSCLC. They found that PTNB and visceral pleural invasion are associated with isolated pleural recurrence in clinical stage I NSCLC. The authors state that PTNB poses a risk of pleural dissemination, and therefore, its application for early-stage lung cancer should be carefully considered.
I think this is a very interesting study. I have the following concerns.

Comment 1
Abstract (Line 12)
This study does not use Cox regression analyses; instead, Fine-Gray subdistribution hazard models are applied.
Reply: Thank you for the comment. We have revised the Abstract to accurately reflect the statistical method used.
(Abstract, Line 11-13) After 1:1 propensity score matching (PSM), risk factors for pleural recurrence were assessed using Fine-Gray subdistribution hazard models. Cumulative incidence rate and overall survival (OS) were also analyzed.

Comment 2
A Fine & Gray model estimates the subdistribution hazard ratio (SHR), not the hazard ratio (HR). Please use the correct statistical terminology. Below are instances where HR has been incorrectly used.

・Line 15-16, Line 121-133

・Table 4, Table 5

・Supplementary table S1 ,S2

Reply: We appreciate the reviewer's observation. We have corrected the term "hazard ratio (HR)" to "subdistribution hazard ratio (sHR)" throughout the manuscript, including tables and supplementary materials.
(Abstract, Manuscript, Table 4-5)

Comment 3
Statistical Analysis
Did you assess the normality of the continuous variables? It is necessary to include the statistical tests used to evaluate normality and their results within the text
Reply: As recommended, we assessed the normality of continuous variables using both the Shapiro–Wilk test and the Kolmogorov–Smirnov test.
(Manuscript, Line 117-8) The normality of the continuous variables was analyzed by using both Shapiro-Wilk test and Kolmogorov-Smirnov analysis.

Comment 4
The definition of pathological tumor size is unclear. It is necessary to specify whether it refers to the total tumor diameter or the invasive tumor diameter.
Reply: We clarified that the pathological tumor size refers to the total tumor diameter. The information has been added to the relevant tables.
(Table 1,2,4,6-9)

Comment 5
AUC (Area Under the Curve) is a useful metric for evaluating the appropriateness of Propensity Score Matching (PSM). Specifically, it is used to assess how well the propensity score model distinguishes between the target group and the comparison group. Therefore, please include additional details on the AUC of the PSM used in this study.
Reply: Thank you. We have now included the AUC value (0.77), which indicates an

acceptable model fit for propensity score matching.

(Manuscript, Line 123-5) We have evaluated the appropriateness of PSM by area under curve metric. Area under curve value was 0.77 which is over 0.7 meaning the propensity score model was well analyzed (Supplementary Figure S1).

Comment 6

Line 103

It is stated that 1,062 patients were included after PSM, but shouldn't it be 1,060 patients instead?

Reply: Thank you for pointing that out. We corrected the number of matched patients to 1,060 (530 per group).

(Manuscript, Line 147-8) Out of 1,733 patients, 1,060 were included after PSM, with 530 patients from each of the PTNB and non-PTNB groups (Figure 1).

Comment 7

Lines 116-120 present the pre-PSM analysis, but the key results are missing. Additionally, the pre-PSM results should not be included in the section discussing post-PSM outcomes. Therefore, either the subheading should be revised or the results of the Fine-Gray subdistribution hazard models before PSM should be moved to an appropriate paragraph.

Reply: Based on the reviewer’s suggestion, we have removed the pre-PSM Fine–Gray model results to improve clarity.

Comment 8

Please add a discussion on the lack of difference in overall survival (OS) between the two groups, as it is a secondary endpoint.

Reply: We added brief discussion noting that overall survival was not significantly different between groups, despite differences in recurrence.

(Manuscript, Line 253-7) Interestingly, despite a higher recurrence rate in the PTNB group, OS did not differ significantly between groups. This discrepancy may be

explained by the effectiveness of post-recurrence treatment. Among patients who experienced recurrence, the majority received systemic therapies such as chemotherapy or TKIs, which likely mitigated the impact of recurrence on long-term outcomes [22].

Comment 9

What treatments were administered for recurrence? Although there was a difference in RFS between the two groups, there was no difference in OS. Could this be because appropriate treatments were provided for recurrence in the PTNB group, resulting in no difference in OS?

Reply: We agree. Among 136 patients with recurrence, 117 (86%) received post-recurrence therapy. In particular, 28 patients in the PTNB group received tyrosine kinase inhibitors. These treatments likely contributed to the comparable overall survival outcomes. We added the additional data in the manuscript.

(Manuscript, Line 164-6) Among the 136 patients who developed recurrence, 117 (86.0%) received post-recurrence treatment. Tyrosine kinase inhibitor (TKI) therapy was administered to 28 patients (29.5%) in the PTNB group and 12 patients (29.3%) in the non-PTNB group (Supplementary Table S1).

Comment 10

How long after PTNB was local pleural recurrence diagnosed?

Reply: Among 17 patients with isolated pleural recurrence after PSM, 13 had undergone PTNB. The median interval between biopsy and recurrence was 14.3 months (range, 7.9–61.0 months; interquartile range [IQR], 12.3–27.7 months). We have described the additional findings in the manuscript.

(Manuscript, Line 154-6) The median interval from biopsy to the diagnosis of isolated pleural recurrence was 14.3 months (range, 7.9–61.0 months; interquartile range [IQR], 12.3–27.7 months).

Comment 11

In the conclusion, it states: "Given the significant adverse effects of PTNB, decisions

regarding this procedure for early-stage lung cancer should be made with careful consideration." However, this sentence is not cited from the Discussion section. Please add a similar statement to the Discussion section.

Reply: We added a corresponding sentence to the Discussion to reflect the cautious interpretation emphasized in the Conclusion.

(Manuscript, Line 264-7) Nevertheless, the evidence for a direct causal relationship between PTNB and pleural recurrence remains circumstantial. The rarity of this event, coupled with the retrospective nature of our study, precludes definitive conclusions. While our findings highlight a potential risk, they do not negate the clinical value of PTNB, especially when non-surgical management is under consideration.

Reviewer B

Thank you for the opportunity to review this manuscript, which investigates the association between preoperative percutaneous transthoracic needle biopsy (PTNB) and pleural recurrence in patients with stage I non-small cell lung cancer (NSCLC). While the topic is clinically relevant and potentially impactful, I have serious concerns regarding the study design, interpretation, and clinical applicability that preclude publication in its current form.

1. Major Concern: Patient Selection and Clinical Inconsistency

One of the most critical issues in this study is the composition of the PTNB group. According to Table 1, patients who underwent PTNB had a markedly higher prevalence of visceral pleural invasion (VPI, 33.5%) and lymphatic invasion (ly, 32.2%) compared to the non-PTNB group. This is clinically counterintuitive.

In current thoracic oncology practice, PTNB is generally avoided in patients with suspected VPI or high clinical T stage, as such cases are more appropriately evaluated intraoperatively via wedge resection with frozen section. In contrast, PTNB is typically

considered for small, peripheral, and radiologically indeterminate lesions where bronchoscopy is not feasible. Therefore, the presence of a large proportion of VPI-positive tumors in the PTNB group raises serious concerns regarding the appropriateness and consistency of diagnostic pathways within the cohort.

Reply: We thank the reviewer for the important comment. We agree that the higher prevalence of visceral pleural invasion (VPI) and lymphatic invasion in the PTNB group appears, at first glance, to be clinically counterintuitive. However, this observation may be explained by the nature of lesions that are typically amenable to PTNB. In general, PTNB is more likely to be performed for peripheral nodules with a larger solid component, which are often classified as higher clinical T stage. Since peripheral location and increasing solid size are both known to be associated with a greater likelihood of VPI, the observed pathologic characteristics in the PTNB group likely reflect the underlying tumor biology rather than a selection bias in clinical decision-making.

To address this imbalance and minimize confounding, we performed propensity score matching based on relevant clinical and demographic variables, including clinical T stage, so that the post-matching comparison more accurately reflects the independent effect of PTNB.

Regarding our institutional approach, we follow the NCCN guidelines, which recommend tissue confirmation—via either surgical or non-surgical biopsy—for nodules suspected of lung cancer, especially when the solid component exceeds 8 mm. While surgical biopsy (e.g., wedge resection with intraoperative pathology) is certainly an option, PTNB is frequently chosen in cases where it is considered safer, more efficient, or less invasive after multidisciplinary evaluation. We have clarified this clinical rationale and institutional decision-making process in the revised manuscript to address this concern appropriately.

(Manuscript, Line 83-96) Diagnostic procedure of PTNB

According to the National Comprehensive Cancer Network (NCCN) guidelines for the evaluation of pulmonary nodules, tissue diagnosis should be considered when a solid or subsolid nodule suspicious for malignancy measures ≥8 mm, especially if the solid

component is present. The recommended diagnostic approach—whether surgical or non-surgical biopsy—should be selected based on lesion characteristics, patient comorbidities, and multidisciplinary evaluation of risks and benefits [18].

At our institution, based on the NCCN guidelines, PTNB is frequently chosen for peripheral lesions with adequate access and solid morphology, particularly when bronchoscopy is not feasible. Although surgical biopsy with intraoperative frozen section is an alternative, several factors influence the decision to proceed with PTNB. These include patient preference (e.g., reluctance to undergo surgery without a confirmed diagnosis), the referring physician's judgment, concerns regarding potential medicolegal implications of operating without pathological confirmation, and logistical considerations. Conversely, in patients with very early-stage disease where PTNB is technically challenging or not feasible, surgical resection with intraoperative diagnosis may be performed following informed consent.

(Manuscript, Line 242-6) We observed that the PTNB group had more advanced clinical T stage, larger tumors, and higher incidences of VPI and lymphatic invasion. These characteristics could partially explain the higher recurrence rates. Given that PTNB is typically performed for peripheral lesions, the higher VPI rate in this group may reflect anatomic proximity to the pleura. Prior research, including work by Inoue et al. [12], has suggested that subpleural location may itself be a risk factor for pleural recurrence.

2. Inadequate Control for Key Confounders

Although the authors implemented propensity score matching (PSM), VPI and lymphatic invasion remained imbalanced post-matching (standardized mean differences of 0.076 and 0.097, respectively). These pathological features are themselves strong independent predictors of recurrence, as shown in both univariate and multivariate Fine–Gray analyses. Therefore, attributing increased pleural recurrence to PTNB without adequately separating the effect of VPI and ly is problematic and risks over-interpreting the causal relationship.

Reply: Thank you for raising this concern. We acknowledge that VPI and lymphatic

invasion remained slightly imbalanced post-PSM (SMD < 0.1). According to statistical convention (Austin, 2009), SMDs below 0.1 are considered indicative of acceptable balance. Nevertheless, we have interpreted the results with caution and have added discussion regarding potential residual confounding.

(Reference added: Austin PC, Stat Med. 2009)

3. Lack of Subgroup Analysis in Low-Risk Cohorts

The central hypothesis—that PTNB contributes to pleural recurrence via needle tract dissemination—can only be supported if the association remains significant in VPI-negative and ly-negative subgroups. However, no stratified analyses were provided. Without such analysis, it is not possible to disentangle the effects of PTNB from those of intrinsic tumor aggressiveness.

Reply: As suggested, we performed subgroup analyses excluding patients with VPI and lymphatic invasion. After PSM, 320 patients per group were compared. While the number of isolated pleural recurrence events was limited (n=4), PTNB remained significantly associated with overall recurrence in this low-risk cohort. We have added detailed descriptions of the analysis, limitations due to event count, and relevant tables in the revised manuscript.

(Manuscript, Line 195-218) Subgroup Analysis: low-risk groups

A subgroup analysis was performed to evaluate the impact of PTNB in patients considered to be at low risk for pleural recurrence. This group was defined as patients without VPI or lymphatic invasion. After excluding cases with either VPI or lymphatic invasion, 332 patients in the PTNB group and 810 in the non-PTNB group were eligible for analysis.

Baseline characteristics in this subgroup differed between the two groups in several variables, including age, surgical procedure, histologic subtype, clinical T stage, and pathological tumor size. Patients in the PTNB group were generally older, underwent fewer sublobar resections, had a higher proportion of non-adenocarcinoma histology, presented with higher clinical T stage, and had larger pathologic tumors compared to those in the non-PTNB group (Table 6).

Following PSM, 320 patients remained in each group, with balanced baseline characteristics across variables (Table 7).

Univariate and multivariate Fine–Gray subdistribution hazard models were applied to identify risk factors for recurrence. In the univariate analysis, PTNB remained a significant independent risk factor for isolated pleural recurrence (sHR = 2.29; 95% CI, 1.19–4.38; p = 0.013), along with pathologic tumor size (sHR = 1.86; 95% CI, 1.50–2.30; p < 0.001). Similar associations were observed for all recurrence types: PTNB (sHR = 2.13; 95% CI, 1.13–4.01; p = 0.019) and tumor size (sHR = 1.80; 95% CI, 1.42–2.28; p < 0.001) were both significant predictors (Table 8). Due to the low incidence of isolated pleural recurrence in this subgroup (n = 5, 0.8%), clinical T stage could not be included in the final regression model (Supplementary Table S2).

For the final multivariate model, clinically relevant variables were included: PTNB, histologic subtype, and pathologic tumor size. PTNB, non-adenocarcinoma histology, and larger tumor size were all independently associated with both isolated pleural recurrence and all types of recurrence (all p < 0.001) (Table 9).

4. Limited Event Counts for Isolated Pleural Recurrence

The number of isolated pleural recurrence events is small (13 in the PTNB group and 4 in the non-PTNB group after PSM), which limits the robustness of the statistical analysis. Conclusions drawn from such sparse data, especially using multivariable models, should be interpreted with great caution.

Reply: We fully agree with the reviewer’s concern regarding the limited number of isolated pleural recurrence events. The incidence of such recurrence is indeed very low, which poses a challenge for statistical analysis and robust conclusions. While our study is based on a single-center cohort, it includes a relatively large number of patients compared to previous single-center studies on this topic. Prior studies have shown inconsistent results regarding the association between PTNB and pleural recurrence, and this variability may in part be due to differences in sample size. Given the rarity of isolated pleural recurrence, larger cohorts may increase the likelihood of detecting a potential association, whereas smaller studies may be underpowered to do so.

Recognizing this, we aimed to include as many eligible cases as possible in our analysis to enhance the reliability of our findings.

5. Overstatement of Causal Inference

The manuscript implies a causative link between PTNB and pleural recurrence, which is not justified given the study's retrospective nature and residual confounding. The phrase "PTNB is associated with pleural recurrence" may be technically correct, but the discussion and conclusion should clearly avoid any implication of causality unless supported by stratified or sensitivity analyses.

Recommendation

Due to the methodological limitations, confounding by indication, and insufficient subgroup analysis, the current manuscript does not provide sufficiently robust evidence to support the stated conclusions. Substantial reanalysis, including stratification by VPI and ly status and detailed justification for diagnostic decision-making, would be required to clarify the true role of PTNB in pleural recurrence.

Reply: We fully agree. To avoid overstating causality, we revised the wording throughout the manuscript. We now state that PTNB is "statistically correlated with" pleural recurrence and emphasize the observational nature of our study. A clear cautionary note was added to the manuscript.

(Abstract, Line 28-9) Preoperative PTNB was statistically correlated with an increased pleural recurrence in clinical stage I NSCLC, even among patients without conventional high-risk pathological features.

(Manuscript, Line 264-7) Nevertheless, the evidence for a direct causal relationship between PTNB and pleural recurrence remains circumstantial. The rarity of this event, coupled with the retrospective nature of our study, precludes definitive conclusions. While our findings highlight a potential risk, they do not negate the clinical value of PTNB, especially when non-surgical management is under consideration.

(Manuscript, Line 275-6) Preoperative PTNB was statistically correlated with an increased isolated pleural recurrence in clinical stage I NSCLC, even in patients without

VPI or lymphatic invasion.

Reviewer C

The authors investigated the correlation between PTNB and the risk of pleural recurrence in patients with clinical stage I NSCLC using PSM. This topic is highly relevant to both interventional radiologists and thoracic surgeons. However, several concerns should be addressed:

1. Please clarify the specific indications for PTNB in this study.

Reply: Thank you. PTNB was performed based on NCCN guidelines and physician discretion. Biopsy was considered for nodules >8mm with a solid component, and modality was chosen under multidisciplinary discussion. This has been clarified in the revised Methods section.

(Manuscript, Line 83-96) Diagnostic procedure of PTNB

2. Multiple statistical tests and analyses have been conducted without adjustments for multiple comparisons, which raises concerns about an increased risk of false-positive results.

Reply: We appreciate the reviewer's concern regarding multiple testing. While formal adjustment for multiple comparisons was not applied, we recognize the risk of type I error and have interpreted secondary findings with caution. A statement regarding this has been added to the limitations.

(Manuscript, Line 270-1) Third, multiple statistical comparisons were performed without formal correction, increasing the risk of type I error.

3. In Tables 4 and 5, the values for "All recurrence" are identical. Could you please verify if this is correct? Furthermore, considering the sample size, the number of covariates included in the multivariate analysis appears to be excessive, potentially

compromising the model's validity.

Reply: We appreciate the reviewer comment. We revised the label to "Any recurrence". To avoid overfitting, we reduced the number of covariates in the multivariable model given the limited number of events. This adjustment allows for a more stable model given the limited number of events and the findings remained consistent. We have updated the manuscript and discussed this revision accordingly.

(Manuscript, Line 179-181) Variables with p-values <0.1 in univariate analysis or with established clinical relevance were included in the multivariate model: PTNB, VPI, and lymphatic invasion.

(Table 5, 9)

4. Several systematic reviews have already examined the association between PTNB and pleural recurrence. Therefore, the novelty of the present findings appears somewhat limited.

Reply: We appreciate the concern. Unlike prior studies that primarily included pathologic stage I cases, our analysis focused on clinical stage I NSCLC, which better reflects real-world biopsy decisions. This distinction is emphasized in the Introduction.

(Manuscript, Line 66-9) Previous research on the relationship between PTNB and pleural seeding has produced inconsistent findings. Many of these studies have relied on pathological staging to select patients, which may not accurately represent the conditions typically seen in clinical practice [15, 16].

(Manuscript, Line 237-41) While rare, pleural seeding has been reported, and several retrospective studies have suggested an association between PTNB and pleural metastasis [15, 16]. These studies, however, have largely relied on pathologic staging and may not fully reflect real-world clinical practice where decisions are made based on clinical staging. In our study, we focused exclusively on patients who underwent curative-intent resection and evaluated outcomes based on clinical staging.