

## Peer Review File

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### Reviewer Comments

A well-structured and important work that addresses a critical topic in pulmonology: tumor tissue is currently, in the era of precision medicine, more of an issue than ever!

The authors acknowledge the crucial limitations, such as small size and single-center experience.

**Comment 1.** Were there any differences in the outcome of IHC when comparing operators (bronchoscopists, skilled vs. newly trained in the technique for cryobiopsy)?

**Reply 1:** Thank you for your question. In our department, all procedures, as a precondition, are performed by or under the supervision of bronchoscopist experts. For your information, we have compared the IHC results between the skilled and newly trained in the technique for cryobiopsy. Of the 40 cases, 15 were performed by skilled bronchoscopists.

For PD-L1, when the IHC results were divided by 0%, 1–49%, and 50–100%, the agreement rate was 93.3% (14/15) by the skilled bronchoscopists whereas 80% (20/25) by the newly trained. For HER2, only one case had a different result when the IHC scores were binarized to 0/1+ and 2+/3+, and that was performed by newly trained. For HER3, when the IHC scores were similarly binarized, the concordance rate of results both by skilled (12/15) and newly trained (20/25) were 80%.

The above results suggest that whether the operator is skilled or newly trained does not significantly affect the IHC results.

To clarify our system in bronchoscopy, we have added the following sentence.

In page 13, lines 16–17: All procedures were performed by or under the supervision of bronchoscopist experts.

**Comment 2.** In four cases (10%), an NSCLC NOS (not otherwise specified) was diagnosed. This is a comparable number to other published studies. I want to invite the authors to comment on why the portion of NSCLC NOS is still relatively high, even though a more significant amount of biopsy tissue (cryobiopsies) was used to establish diagnosis and subtyping of lung cancer.

**Reply 2:** Thank you for your comment. The pathological diagnosis of lung cancer is based on the latest WHO classification<sup>1</sup>. Morphologically NSCLC without a clear differentiation tendency to adenocarcinoma or squamous cell carcinoma, supplemented by immunohistological staining with TTF-1 for the former and p40 for the latter, is finally separated into favor Ad (TTF-1 positive), favor Sq (p40 positive), and NSCLC NOS (both negative).

Cryobiopsy specimens are certainly considered more favorable for morphological diagnosis than forceps biopsy specimens due to their larger size and less crush artifacts. However, the diagnosis of NOS is eventually made by the IHC above; thus, it would be reasonable results that NOS has been diagnosed with the comparable proportion to previous reports.

1. Nicholson AG, Tsao MS, Beasley MB, et al. The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015. *J Thorac Oncol.* 2022;17(3):362–387.