Peer review file
Article information: https://dx.doi.org/10.21037/jtd-21-137

Reviewer A

Comment 1: *Unfortunately, I do not see the clinical value in predicting VPI during surgery as the lung will be removed and the pleura will be evaluated under the microscope regardless of the result of the microscopy. If you could predict this pre-op, it may be useful to guide neoadjuvant therapy. What is the actual added value to confocal microscopy in this setting?*

Reply 1: We are considering using the intraoperative CLE diagnosis of VPI to determine the indication of sublobar resection for small-sized peripheral NSCLC. If VPI was diagnosed by CLE, the technique would be to change lobectomy and standard lymph node dissection from sublobar resection, because lymph node metastases are more common in patients with VPI, which is thought to be one of the reasons of the worse prognosis.

We have added the following sentence to the Discussion.

Changes in the text: If VPI was diagnosed by CLE, the technique would be to perform lobectomy and standard lymph node dissection without performing sublobar resection, because lymph node metastases are more frequent in patients with visceral pleural invasion (6), which is thought to be one of the reasons of the worse prognosis (p. 17, lines 294–297).

Comment 2: *Also, the sample size is extremely small, making it difficult to see the actual sensitivity go the test.*

Reply 2: We agree with this comment. We have added the following sentence to the Discussion.

Changes in the text: Therefore, to apply this method clinically, performing a multicenter clinical research with a large number of cases is required (p. 20, lines 357–359).

Thank you again for your comments on our paper. We hope that the revised manuscript is now suitable for publication.

Reviewer B

Comment 1: *Most importantly, the size of a tumor in this study was too big. VPI could help determine the treatment strategy when the tumor is small. Once the tumor is big enough, it may not matter whether the VPI is present. Therefore, it is essential whether the device can diagnose VPI occurring in small-sized lung nodules, and additional studies are required in those subsets of patients. Furthermore, diagnostic criteria and their accuracy should be present according to the size of a tumor.*

Reply 1: We agree with this comment. We have added the following sentence as a limitation of the study to the Discussion.

Changes in the text: Moreover, the study included NSCLC patients with a relatively large tumor although intraoperative CLE diagnosis of VPI was expected to be applied to the indication of sublobar resection for small-sized peripheral NSCLC (pp. 20–21, lines 359–361).

Comment 2: *In a similar vein, clinical characteristics of cases showing false negative or false positive results should be presented. For example, in case of severe adhesion or tight adhesion which was not expected preoperatively, could this method differentiate pleural invasion or pleural thickening?*

Reply 2: Thank you for the insightful comments. Though we did not encounter the cases of severe adhesion or tight adhesion, we have a few findings that seemed to cause the false-positive cases. Therefore, we have added the following sentence to the Discussion.

Changes in the text: Though the sensitivity of CLE observation in diagnosing VPI was high, the specificity was relatively low. The reason why there were few false-positive cases was that the autofluorescence-positive network structure was hardly observed at the visceral pleura that showed anthracosis or pleural thickening caused by obstructive pneumonia (p. 20, lines 352–356).

Reviewer C

Comment 1: *There is no information about blinding the CT results to the observers.*

There is also no information about blinding the information between the observers. It is an important issue because assessing the intraoperative view after the assessment of CT may be biased.

Reply 1: We fully agree with this comment. Accordingly, we have revised the sentence in the Materials and Methods as follows.

Changes in the text: Three thoracic surgeons blinded to clinical information including CT findings and pathologic diagnosis of cases viewed the videos and independently determined the defect ratio of autofluorescence-positive structures based on a scale of five (0%, 25%, 50%, 75%, 100%) (p. 12, lines 194–196).

Comment 2: *Line 264. The information about difficulties in the assessment of VPI in CT is supported by a citation of a paper comparing different types of intraoperative assessment. Please consider finding a more relevant paper to eventually support this thesis.*

Reply 2: Thank you for the insightful comments. We have cited several papers and compared them with our results to show the difficulty of preoperative and intraoperative VPI diagnosis.

Changes in the text: In our ex vivo study, the sensitivity, specificity, positive and negative predictive values, and accuracy for VPI using defect ratios of autofluorescence-positive structure cutoff of ≥50% were 83.3–100.0%, 57.7–73.1%, 35.3–41.7%, 95.0–100.0%, and 75.0–78.1%, respectively. Furthermore, in the validation study, the sensitivity, specificity, and accuracy were 100%, 83.3%, and 86.7%, respectively. There have been several studies about preoperative VPI diagnosis using CT findings. The sensitivity, specificity, positive and negative predictive values, and accuracy for VPI using CT findings were 36.4–45.0%, 91.0–92.9%, 76.2–94.0%, 36.0–69.6%, and 57.0–71.0%, respectively (18,19). Tanaka et al. reported fluorine-18-fluorodeoxyglucose uptake for risk stratification of VPI of lung adenocarcinoma, and the sensitivity, specificity, positive and negative predictive values, and accuracy for VPI using maximum standardized uptake value were 63.6%, 88.1%, 51.9%, 92.3%, and 84.0%, respectively (20). As regards intraoperative VPI diagnosis, the sensitivity, specificity, and accuracy using an autofluorescence endoscopy system were 83.3%, 73.7%, and 76.0%, respectively (17). Kitada et al. reported an autofluorescence observation method after oral intake of 5-amino-levulinic acid with the sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 58.0%, 63.1%, and 100%, respectively (21) (pp. 17–18, lines 298–313).

Newly added references 18-21

18. Hsu JS, Jaw TS, Yang CJ, et al. Convex border of peripheral non-small cell lung cancer on CT images as a potential indicator of pleural invasion. Medicine (Baltimore) 2017;96:e7323.

19. Hsu JS, Han IT, Tsai TH, et al. Pleural Tags on CT Scans to Predict Visceral Pleural Invasion of Non-Small Cell Lung Cancer That Does Not Abut the Pleura. Radiology 2016;279:590-6.

20. Tanaka T, Shinya T, Sato S, et al. Predicting pleural invasion using HRCT and 18F-FDG PET/CT in lung adenocarcinoma with pleural contact. Ann Nucl Med 2015;29:757-65.

21. Kitada M, Ohsaki Y, Yasuda S, et al. Photodynamic diagnosis of visceral pleural invasion of lung cancer with a combination of 5-aminolevulinic acid and autofluorescence observation systems. Photodiagnosis Photodyn Ther 2017;20:10-5.

Comment 3: *Please provide information about the time of intraoperative procedure and how it affected the time of surgery.*

Reply 3: Accordingly, we have added the following sentence to the Results.

Changes in the text: It took about 5 minutes to observe the pleural surface by CLE and diagnose VPI (p. 16, lines 279–280).

Comment 4: *Please provide a figure or preferably photo of the device, how it is set up during the operation. What are the ergonomics of the device?*

Reply 4: Thank you for the comment. We have added Figure 3.

Changes in the text: Figure 3. The CLE probe progressed into the thoracic cavity and the tip of the probe was guided to the site of pleural change (p. 28, Figure 3, highlighted version).

Comment 5: *The most important issue is the lack of effective comparison of the methods. Please assess the sensitivity, specificity, NPV, and PPV of the investigated methods, CT image, direct vision. On the basis of this table, it would be possible to estimate the true meaning of the study. The comparator should be a pathological invasion of visceral pleura (PL1). This is essential for the eventual implementation of the technique.*

Reply 5: We fully agree with this comment. We have added the data of the sensitivity, specificity, NPV, and PPV of the investigated methods, CT findings, and direct vision. Moreover, we have added Table 4.

Changes in the text:

Diagnosis of VPI by CT findings and intraoperative observation under white light

Three thoracic surgeons who participated in a study of VPI diagnosis by CLE evaluated VPI of the same 35 patients by CT findings and intraoperative observation under white light. For VPI diagnosis by CT findings, the surgeons independently reported that VPI was positive when the tumor showed the following CT findings: pleural contact, pleural thickening, solid proportion >50%, and lesion size >20 mm. For VPI diagnosis by intraoperative observation under white light, the surgeons were blind to the CT findings of the cases. A 10-s video of each case was played to provide the surgeons the best view showing the pleural surface where the tumor was located, inducing morphological change. The surgeons revealed that VPI is positive when the pleura showed the following CT findings: whitish change, granular change, and hypervascularization of the pleural surface (pp. 12–13, lines 201–211).

Diagnosis of VPI by CT findings and intraoperative observation under white light

The sensitivity, specificity, positive and negative predictive values, and accuracy for VPI using CT findings were 83.3%, 62.1–75.9%, 31.3–41.7%, 94.7–95.7%, and 65.7–77.1%, respectively. The sensitivity, specificity, positive and negative predictive values, and accuracy for VPI of intraoperative observation under white light were 66.7–83.3%, 69.0–72.4%, 30.8–38.5%, 90.9–95.5%, and 68.6–74.3%, respectively (Table 4) (pp. 15–16, lines 264–269).

Comment 6: *Line 87-88, lines 260-261. I do not agree that the prognosis of patients in stage IB NSCLC is poor. Please review this part of the manuscript. Please consider citing more classic papers on NSCLC prognosis depending on the pathological stage.*

Reply 5: Thank you for your comment. We have changed ref. 7.

Changes in the text:

7. Lakha S, Gomez JE, Flores RM, Wisnivesky JP. Prognostic significance of visceral pleural involvement in early-stage lung cancer. Chest. 2014 Dec;146(6):1619-1626. (reference 7).