Peer Review File

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Reviewer A

The authors present a large case series detailing their experience with using ICG dye marking of pulmonary nodules prior to VATS. The lesions are quite small, without a bronchus sign, peripheral, and non-solid in nature. The manuscript is quite informative and may be improved further with some additional details and clarifications as below.

Methods
What criteria were used to determine that the "preoperative chest CT revealed that the lesions were challenging to localize or identify with observation or palpation during surgery"? Please expand upon this further as this could be quite subjective.

Reply: thanks for your comments. The criteria would be: 1. the surgeon could not reach the nodule with the finger; 2. nodules away from the visceral pleura that are difficult to observe or touch; 3.the nodules were mainly ground glass opacity, which were difficult to identify by touch. We have made a specific description in the Methods section.

Changes in the text: we have modified our text as advised (see Page 5, line 90-93).

Was the navigation procedure done with a single lumen ET tube in place or the double lumen ET tube that would be needed for the following VATS surgery? Did both the navigation and VATS occur in the same OR? Please clarify if two intubations occurred and where the procedures occurred as this could impact timing and logistics. The later is mentioned in the discussion, but I would also clarify in the methods.

Reply: thank you so much for your comment. The navigation and VATS occurred in the same OR. Navigation and surgery are usually performed in our center under spontaneous respiratory anesthesia with laryngeal mask. Intubation is not required in the majority of cases. In order to ensure the safety of patients, anesthesiologists will prepare a double-lumen endotracheal tube to cope with accidents (such as unstable vital signs, large amplitude of mediastinal swing during surgery) which was also illustrated in the Methods section. In this study, only one patient was given double-lumen endotracheal intubation due to intolerance to non-intubation anesthesia.

Changes in the text: we have modified our text as advised (see Page 6-7, line 115-116/135-136).

How were the distances chosen for table 1 for the localization accuracy scoring system? Was a validated system already in place and if so are there references? Was consensus from the authors used? A different method? Details on this would be very informative.

Reply: thanks for your suggestion. Unfortunately, there is no literature to clarify the distance standards. This scoring system was decided jointly by all authors.

Discussion
It is unclear, but how many nodules, if any, in this cohort also underwent methylene blue staining? Or does this statement reference the authors' prior experience only and not this paper? Did this impact the accuracy scoring system? Please clarify.

Reply: thanks for the suggestion. All cases in this study were stained with ICG, which did not bring any trouble to the observation or the scoring system. The methylene blue staining experience mentioned in this paper came from the previous clinical practice and did not involve this study.

In Table 2, please provide definitions for AAH, AIS, MIA, IA for readers unfamiliar with these terms. These are listed under Table 3.

Reply: thanks for the suggestion. We have made the essential modifications in Table 2 and Table 3.

Changes in the text: we have modified our text as advised (see Page 23-24).

Reviewer B

Zhang et al have provided an excellent paper regarding the utility of ICG in identifying small pulmonary nodules via navigational bronchoscopy to facilitate minimally invasive sublobar surgical resection.
The paper is well written and methodologically sound. The sample size is adequate and the results are impressive, with an extremely high rate of success, and no complications.
I think this paper will be an excellent addition to the literature and is worthy of publication. I do have a small number of minor points which should be considered:
1. The footnote at the bottom of Table 3 should be incorporated into the footnote for Table 2, as it is providing explanations for the pathology abbreviations

Reply 1: thanks for the suggestion. We have made the essential modifications in Table 2 and Table 3.

Changes in the text: we have modified our text as advised (see Page 23-24).

2. In table 3 you state that around 88% of patients scored either 4 or 5 in terms of tracer accuracy (i.e. all <15mm), yet in the text you state that the average distance from tracer to nodule was 33mm. This doesn't seem to correlate correctly.

Reply 2: thanks for your suggestion. We also noticed this problem and had explained it in the

discussion section (page13, lines 248 to 256). When there is no bronchus leading to the lesion, we select the nearest point of the adjacent bronchus for localization. The bronchial ends are far from the lesion, but a certain location in the bronchus may be close to the lesion which may result in a mismatch in distance and accuracy score.

3. Table 4. Comparing 4 groups with a Chi square test is not ideal. Given that you state that a score of either 4 or 5 is deemed accurate, perhaps you should consider repeating the statistical analysis by comparing a group who scored either 2 or 3 with a group who scored either 4 or 5. You would then only be comparing two groups, which would make your statistical analysis much more meaningful.

Reply 3: thanks for your suggestion. We have made pairwise comparisons before writing the article. The data was supplemented in the Appendix.

Changes in the text: we have modified our text as advised (see Page 20).

Reviewer C

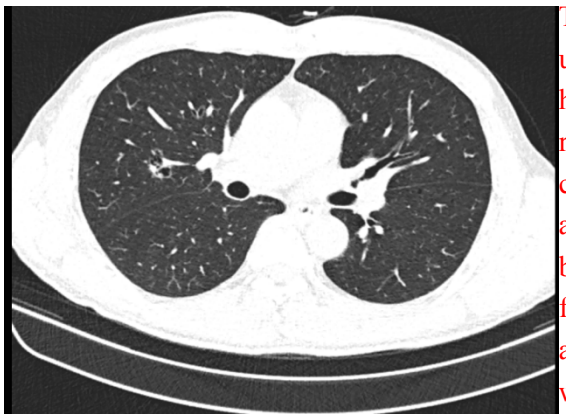
Good job on this topic. However, I did not hear your machine brand. Please give me the picture and the details about the ENB. You better provide the video about how you do it. Moreover, I don't quite understand table 5, and I believe so do our readers. Please give me the details about how the numbers being calculated. Last, there is patient undergoing lobectomy. Please give me the image and describe the clinical situations which we are curious.

Reply: thanks for your suggestion.
1. We have used the navigation system in previous studies (see References: 12, 21), and the navigation details and operations have been published as supplementary data (see References: 12, available online: http://www.asvide.com/watch/32892). Unfortunately, the video cannot be cited in the text for the time being because it has not been given permission by the copyright owner.

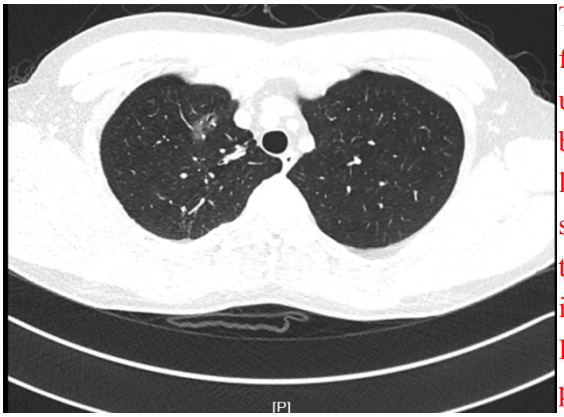
2. The data in Table 5 were generated during the calculations of the ordinal logistic regression analysis. In the calculation process, the software will generate some new variables, such as: estimated response probabilities, predicted category, predicted category probability, actual category probability. Finally, the prediction accuracy was obtained by comparing the frequency of predicted category with the frequency of actual category.

3. Three patients underwent lobectomy in this study.

This patient was a man in his 70s, and his CT scan revealed two nodules in the apical segment of the right upper lung, with long diameters of 1.6 cm and 0.7 cm, respectively. The larger one was considered to most possibly be lung cancer, and the smaller one was proposed to be tumor or inflammatory granuloma. Intraoperative frozen section revealed that the larger nodule was invasive adenocarcinoma, and right upper lobectomy was performed consequently.



The patient was a middle-aged man who usually had symptoms of chest pain and a history of bladder malignancy. CT scan revealed a cyst in the right posterior segment, considering lung cancer associated with cystic airspaces, and the infection around pulmonary bullae could not be ruled out. Intraoperative frozen section revealed invasive adenocarcinoma of the lung, and lobectomy was performed eventually.



The patient was a middle-aged male who was found to have a ground-glass nodule in the right upper lobe in physical examination 1 year before surgery, which was considered to be lung cancer. Follow-up CT showed no significant change in the size of the nodule, but the patient's body weight decreased nearly 5kg in the past 3 months before surgery. Intraoperative frozen section revealed pulmonary invasive adenocarcinoma, and the lobectomy was performed.

Reviewer D

This is an original study reporting on preoperative ENB-guided localization of pulmonary nodules by ICG injection for minimally invasive surgical resection. In this article, the authors retrospectively analyzed data on procedures where ENB was used to dye mark a nodule prior to surgical resection. They found that this method was an accurate and effective method to help find small pulmonary nodules during minimally invasive surgery.

General Comments:

This is a well-documented study on objective findings during ENB guided localization of pulmonary nodules prior to surgical resection.

Questions/Comments:

1. Could the authors please comment whether any additional bronchoscopic localization confirmatory procedure was utilized (ie radial probe US or Fluoro)? If not, would this have been helpful? CT-body divergence of ENB may affect the localization accuracy.

Reply 1: thanks for your suggestion. We did not use any other bronchoscopic localization confirmation procedures. These procedures were helpful, but they might also increase the economic burdens on the patients. We have no relevant experience in this area.

2. Could the authors please clarify "successful" localization (ie is this term being used only for accuracy scores of 4 and 5)?

Reply 2: thanks for your suggestion. Successful localization indicates the following: the airway condition shown by the navigation system matches the actual situation, and we can locate through the optimal route designed by the navigation system. There is no inability to locate due to airway occlusion, absence of airway, etc.

Changes in the text: we have modified our text as advised (see Page 7, line 130-132)

3. Could the authors note whether the dye marking was noticeable during surgery if applied further from the pleura (they comment on better penetration of ICG compared with methylene blue)? What was the distance from the pleura where it was difficult to observe the dye? If unable to observe the dye – could the authors comment on what steps were taken to localize the lesion during surgery?

Reply 3: thanks for your suggestion. It can still be visualized when staining nodules away from the pleura. Of course, the closer to the pleura the nodule is, the more obvious the tracer can be seen. In our previous experience, the dye will become dimmer in visualization when the nodule is more than 2cm away from the pleura. If there was no tracer shown during the procedure, we would locate the nodule according to the three-dimensional reconstruction providing by the navigation system as well.