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Reviewer A

I have reviewed this article many times in recent days. The method of sandwich marking technique using CBCT in the hybrid-OR for localization of small peripheral pulmonary nodule. It is an effective and safer method for localization, avoiding the lethal complication of air embolism from the method of needle dye marking or hook-wire method.
I have some questions about your novel technique.

Comment 1. How do you determine the distance between target and metal clip in the collapsed lung? In Fig 3. The distance between the target and metal clip is more than 30 mm in the expansion lung for CBCT confirmed.

Reply 1: We measured it refer to the length of distal end of forceps for thoracoscope under half ventilation of the affected lung, and confirmed it through CBCT scanning afterward. It is possible to repeat the process until achievement of marking.

Comment 2. How many cases required for performing the sandwich marking technique to achieve the optimal CBCT scan times? I also very interested the case of requiring 8 times CBCT scan. Can you explain it?

Reply 2: We performed CBCT scans 8 times for patients with multiple pulmonary metastases from hypopharyngeal cancer. Wedge resection was performed twice for 2 separate lesions in the right lower lobe by the sandwich marking method. Initial scan was done preoperatively in the hybrid operating room, and 2nd one was failed to demonstrate the CBCT image because artificial athelectasis of the right lung by the anesthesia department resident. Unfortunately, she failed to keep full respiratory expansion of the affected lung again. It was purely technical failure by rookie medical doctor. After 4 times CBCT scans, two pulmonary lesions required further four times scan due to confirm each right locations in the lower lobe of the lung. This was 31th case in the study. We guess that it could be possible to localize multiple lesions less often scan times now because we had enough experiences in this technique in both Department of Surgery and Anesthesiology.

Comment 3. In Table 2. about pathological diagnosis (other: 12.8%). Can you explain it ?

Reply 3: Yes. “Other” included intrapulmonary lymphnodes, Pulmonary epithelioid hemangioendothelioma, Benign metaplastic lesion, Pulmonary capillary

hemangiomatosis, and Localized pneumonia with focal organizing pneumonia in pathology.

Some errors required revision listed as below.
Line 40: tow

We corrected it (Page 3, Line 39)

Line 165: MIL( )?

We defined the abbreviation “MIL (a mixture of indigo carmine and lipiodol)” at the line 79 (Page 4, Line 75). This abbreviation was used by Hasegawa et al. who reported the utility of the dye marker (J Vasc Interv Radiol 2019;30:446-52).

Line 209: 7?

We deleted it.

Congratulation! This sandwich marking technique is an effective and novel method for intra-operative localization in hybrid-OR.

In my opinion, the method of sandwich marking technique is worthy to be published after reply my questions.

Reviewer B

This paper presents a marking method for nodule resection under video-assisted thoracoscopy, based on the intraoperative CBCT localization of metal clips surrounding the nodule(s) to resect. The paper is clear and reads well. The clinical study is clearly described, on a large cohort of 109 nodules on 90 cases. The results are excellent, with a 100% guaranteed negative margins and no particular complications, and a limited radiation dose in comparison to a preoperative marking procedure. While the paper is overall very good, important details about the procedure and the visibility of nodules must be added, as well as more informative figures. I really think the points below can be addressed, which would greatly improve the quality of the paper before publication.
Detailed comments

- line 92: a more thorough review is needed here, as other groups have used CBCT for VATS. For example, Rouze et al. 2016 used a single CBCT and then fluoroscopy for markers-less localization of small peripheral nodules; there might be others. Recently, several research studies also proposed registration methods evaluated on animals

(Nakao et al. 2019, 2020) or retrospective VATS cases (Alvarez et al 2021).

Reply 1: We added some sentences for a more review (Page 6, Line 91-104).

- section starting at line 118 (but the Reply may not be in this §)
One of the main potential limits is the visibility of all nodules in the CBCT images. While peripheral nodules should not be too affected by atelectasis, the density of parenchyma still increases with the pneumothorax which can severely hinder the nodules visibility. Small (up to 2mm in your study) or subsolid nodules (ground-glass opacity) can be very barely visible - or even not visible at all. This visibility problem should at least be discussed. CBCT images of the difficult cases could also be shown.

Reply 2: It was very important to inflate the affected lung with no atelectasis during CBCT imaging. In some cases, the anesthesiologists failed to do it, but re-challenge in the second scanning. Although the scan times were increasing if the anesthesiologists failed to do it, they were able to repeat it as much as we wanted. So, it was no need to have the concern that small nodules could be hindered in atelectasis, but instead it would be a problem to increase patients' radiation exposure. According to your suggestion, we remade Figure 2 demonstrating that very small ground glass opacity was imaged by CBCT (Figure 2B-D).

Other general questions regarding the CBCT:
- what is the field of view (FOV) of the volume, in mm, as well as the image resolution?

Reply 3: 250.49*193.51*250.49mm3 FOV

- is the lung always entirely visible, as well as all clips? If not, was is it difficult to position the CBCT/patient correctly to ensure that the nodule and clips lie within the FOV?

Reply 4: The lung was not always entirely visible, but all clips were visible. The surgical table was moved and adjusted before scanning at the proper position where was confirmed with fluoroscopy function of CBCT. For example, if the target lesion was located at right lower lobe of the lung, the surgical table was moved at the level of the lower lobe. In this case, it was not needed to scan the right upper lobe. And, if 1st scan failed to detect the target lesion, you can re-try 2nd scan to ensure the position of the CBCT/patient correctly, or nodule/clips.

- line 129: what is the goal of the skin marking procedure: to position the surgical ports optimally, above the nodule position before pneumothorax, as explained line 145? This could be illustrated, in Fig.3 for instance.

Reply 5: The purpose of the skin marking is that we can predict the position of the

target lesion in referring to it during VATS. And, we can select the optimal port sites for VATS. Sorry, we did not understand the meaning of “before pneumothorax”, you wrote. Sandwich marking technique never require any percutaneous needle puncture for marking. And, full expansion of the affected lung should be kept during CBCT scanning. In other words, “pneumothorax” was not needed in this technique. That is why we did not explain it by using any additional figures.

Did you assess the quality of this pre-positioning? The nodule may shift considerably from its initial position due to the pneumothorax.

Reply 6: As we describe it above, we never need “pneumothorax” in this technique. Our technique is completely different from other techniques reported by Rouze et al, Alvarez et al. (13, 14). We scanned the fully expanded affected lung with metal clips.

- line 137 “to identify the positional relationship among the clips and the pulmonary target lesion (Figure 2B)”: the link between this sentence and the image is unclear. What exactly is being displayed in 2b? What is the meaning of the yellow star (below the nodule)? An image (resliced if needed) with the nodule and at least one skin clip would be interesting here.

Reply 7: We changed the picture in Figure 2B.

- line 150 “… Ideally, the clipping was performed by maintaining a distance of approximately 30mm”: since the nodule moves with the pneumothorax, at what rate can you achieve this target placement of the clips? As stated line 275, this is “The most important aspect” of your method. Was this 30mm criterion the reason for repeated CBCT scans? What was your procedure if clips were too close to the nodule? What was your procedure if clips were too far from the nodule or to the side? Did you place additional clips?

Reply 8: Sandwich marking technique need no pneumothorax. We believe 30mm could be enough for surgical margin from any pulmonary malignancy. If the distance is much closer, the risk of local recurrence could be increasing, we guess. We placed additional clips if clips were too close or far from the target lesion, and scan repeatedly until confirming the right distance.

- Was the visceral pleura harmed while placing the clips? Especially, did you encounter significant bleeding or air leakage?

Reply 9: We found a little bleeding or air leakage in few cases, especially which had emphysematous lung. Conversely, there were several cases in which a clip had come off during full expansion of the affected lung. A clip tends to drop when the anesthesiologist inflated the lung. So, it is important to clip sufficiently against power of expansion of the lung. If the lung was injured by clips, we sutured it or resected it

by endostapler.

- Figure 3d suggests that all clips were removed with the wedge resection: is this guaranteed (in which case this should be clarified in the manuscript)? If more clips had to be placed for a correct localization, is the wedge larger than usual to ensure the removal of all clips? If some clips were placed on non-resected tissue, was the visceral pleura harmed on these locations after their removal?

Reply 10: We removed all unnecessary clips by the thoracoscopic forceps, and repaired the injured visceral pleura if needed. And, we performed wedge resection of the lung including the target lesion and all clips. We added the sentence “All unnecessary clips were removed by the forceps before wedge resection, and wedge resection was performed including all marker clips. As needed, any injured visceral pleura was repaired by suture or resected by endostapler.” (Page 8, Line 167-170).

- A figure with post-pneumothorax CBCT slices showing the nodule and clips is clearly mandatory here.

Reply 11: We explain Sandwich marking technique again here. It does not require pneumothorax. Probably, you kindly study other publication of CBCT marking, but which could be different from our method regarding no use of the pneumothorax CBCT images.

- line 155 “… where should take distance enough as surgical margin from the target lesion.”: the last part of this sentence is unclear.

Reply 12: We rewrote the sentence to “It was noted carefully to keep the distance, about 30mm, between the target lesion and a marker clip due to ensure surgical margin” (Page 8, Line 166-167).

- line 158: very interesting study of the skin radiation dose, that is well discussed later (l. 279) Since dosimeters are placed opposite the operated lung, are they in the FOV of the CBCT? If not, how many of the 2D projections are accounted for to compute the dose?

Reply 13: Some dosimeters were within the FOV, the others were out of the FOV. Depends on location of the target lesions (upper/middle/lower lobe), the number of dosimeters out of the FOV was different. For example, 5th dosimeter could be out of the FOV in the case having the target pulmonary lesion in apex of the upper lobe. Sorry, we had no data in detail which cases had all dosimeters within the FOV, or not.

Sentence “Theoretically, skin exposure dose, including that of scattered radiation, was measured; it was more than the exposure dose of CBCT” suggests that the measured dose is more important than within the CBCT FOV. If that is correct, would you have

a reference for this point?

Reply 14: Here, we would like to emphasize one important fact that the patient receives the unnecessary radiation including scattered radiation out of the FOV*. In other words, the patient radiation exposure consists of not only within the FOV but also out of the FOV. To explain this point more clearly, we rewrote the sentence "Theoretically, those dosimeters measured skin exposure dose, including that of scattered radiation not only within the field of view (FOV) but also out of the FOV in CBCT". (Page 8, Line 176-177))

*Lee CH, Ryu JH, Lee YH, Yoon KH. Reduction of radiation exposure by lead curtain shielding in dedicated extremity cone beam CT. Br J Radiol. 2015 Jun;88(1050):20140866. doi: 10.1259/bjr.20140866. Epub 2015 Mar 26. PMID: 25811096; PMCID: PMC4628457.

- 190 Characteristics of nodules: complete characterization. Could you just specify the number of very small nodules, and whether any ground-glass opacity nodule was included?

Reply 14: We added results of Consolidation/Tumor Ratio on MDCT in Table 2. There were 53 solid nodules (49.6%), 39 sub-solid (35.8%), and 17 pure ground-glass opacity (15.6%).

- 201 tumor size: how were the lesions measured in the CBCT, by their larger axis? Was this measurement hindered by the (potentially poor) nodules visibility?

Reply 15: We should note that all target lesions were suspected of being malignancy. In other words, for example, pure GGN with a maximum diameter of 2mm was not be included into this study because such a lesion was not suspected of being malignancy. We measured the larger axis of each lesion. Smallest pulmonary nodule was 2mm in solid nodule, 5mm in sub-solid nodule, and 4mm in pure ground-glass opacity. We were very surprised the fact that CBCT detected only 4mm pure GGO.

- Fig 5: if possible, use the same axis range for the 3 bar plots (although the pathology measures are less ventilated)

Reply 16: Sorry, it is very difficult for us to re-scale for the 3 bar plots on R software.

- line 236 " In 2013, Uneri et al. …": see above, more recent works were recently published.

Reply 17: Yes, we know. We think it is very important who first reported this CBCT-guidance system in the world. As far as we know, Uneri et al. were the first researchers who published animal experimental results in 2013. That is why we cited

the paper.

- line 252: the average number of CBCT scans was 2.7, up to 8. How many cases could be treated with two scans only, one for the skin marking and one for the sandwich marking? What were the reasons for additional scans: multiple nodules, FOV, increase of the dose for a better nodule visibility, placement of additional clips, … ? There is a clear lack of information regarding this point in the paper. The authors should explain this more clearly.

Reply 18: 46 cases had 2 CBCT scans (51.1%). There were some reasons why multiple CBCT scan (>2 times) was required, including thoracic adhesion, multiple targets, failure of sandwich marking at first marking, failure of maximum inspiratory ventilation of both lungs, marker metal clip fall off, or additional resection for ensuring surgical margin. Then, we described it like “There were some reasons why multiple CBCT scans (>=4 times) was required, including failure of sandwich marking/refine localization (n=8), multiple target lesions (n=5), and/or thoracic adhesion/refine localization (n=3)”. (Page 12, Line 273-276)

In certain cases, e.g. to refine localization, would it be possible to use 2D views or fluoroscopy instead of a full 3D CBCT scan?
Reply 19: Yes, we can use 2D views or fluoroscopy anytime in a Hybrid-OR.

- line 271 “This is the first technique that enables location and resection of impalpable pulmonary nodules…”: always be cautious with this kind of claim. For example, see Rouze et al. 2016 (doi: 10.1093/icvts/ivw029). While their study was more limited with 8 patients only, these authors also presented a CBCT-based method for nodule localization and resection; there might be other papers such as reference 19. This sentence could be better hedged.

Reply 20: Thank you for your positive suggestion. Accordingly, we corrected the sentence, below.
“This is the robust technique that enables location and resection of impalpable pulmonary nodules even in case of severe intrathoracic adhesions.” (Page 13, Line 298-299)

- lien 276 “We believe it can be technically easy for surgeons to learn…”: could you mention how many surgeons participated to this study (one for each center, or more?)

Reply 21: There were 9 surgeons who participated to this study. Furthermore, when I say in detail, one surgeon (Y.S.) is the technical instructor who taught other surgeons, including 3 young surgeons who was training. We added the sentence “In this study, there was one experienced surgeon (Y.S.) developed this technique and gave instruction in it to other eight surgeons, including three surgical residents.” (Page 13, Line 305-307)

- Table 1: I understand that 2.7 were the average number of CBCT acquisitions. Did you estimate the surgical time dedicated to the setup, patient positioning, and image acquitions? In 256 you mentioned a surgical of more than two hours, but could you report the additional time of your study with respect to a standard procedure.

Reply 22: Sorry, we can not estimate time necessary for CBCT imaging during surgery because it was impossible to divide it clearly into surgery time and CBCT time. There were time of preparation for CBCT scan, time of CBCT scan, and time of preparation for restart of operation. We guess 5-10min necessary for each CBCT scan.

Reviewer C

This is a retrospective review of the authors' experiencing performing what they call the "sandwich technique" to identify the location of small lung lesions that are to be resected with a wedge. They combine the use of clips and cone beam CT to locate these lesions and perform their wedge resections.

Major comments:

1. Please describe how many of these lesions were solid, subsolid or ground glass

Reply 1: We added data about C/T ratio (Consolidation/Tumor Ratio on MDCT) in Table 2, below.

Solid (1)	53 (49.6)
Sub-solid (0<, <1)	39 (35.8)
Pure ground glass nodule (0)	17 (15.6)

And furthermore, we added the sentence “Consolidation/Tumor ratio (C/T ratio) indicated 53 solid nodules (49.6%), 39 sub-solid nodules (35.8%), and 17 pure ground glass nodule (15.6%) in Table 2” (Page 10, Line 210-212).

2. Please describe how many were palpable (or not palpable).

Reply 2: We added the sentence “And then, 41 lesions (37.6%) were palpable” (Page 10, Line 212).

3. How often was the placing of the clips correct and had the lesion in between them with the first cone beam CT? How often did you need to ree-position the clips after obtaining the cone beam CT? How far were the initial clips from the target lesion when you did this first CT? none of these questions are clear, and it seems like every time you place the clips and did a CT, the lesion was in between your clips (lesion found in 100% of cases). If that is the case, you do not even need to place the clips, you are always right and can proceed with your wedge.

Reply 3: We were able to place the clips sandwiching the lesion in between them with

the first CBCT in 46 patients (51.1%). We needed to re-position the clips in around 35 cases (around 40.0%). The distance between the initial clips from the target lesion was not able to be measured technically because we removed it after 2nd clips were placed in thoracic cavity. In our experiments, 3 times challenges could be enough for right placing of metal clips sandwiching the pulmonary lesions in almost all cases, we think.

4. In the abstract section, your main results should be your primary endpoints: localization with clips/CBCT, rate of R0 resection and radiation. Remove the correlation coefficient of tumor size by all modalities.

Reply 4: We deleted the sentence “To compare the size of pulmonary lesions, the correlation coefficient was 0.82 between cone-beam computed tomography and multi-detector computed tomography, and 0.66 between cone-beam computed tomography and pathology, respectively” (Page 3, Line 50-53).

5. I do not understand the meaning of the correlation coefficient of size amontg CBCT. MDCT and path. There is always some discrepancy, and nobody really cares, because for the final TNM you will use the pathology which is more accurate. This does not add to the manuscript and I would remove it. It will also depend on the characteristics of the lesion (if they have a GGO component).

Reply 5: Sorry, we have different opinion against what you described, above. As you know, the tumor size in pathology was most accurate. We think some researchers would like to know the relationship between pathological tumor size and radiological tumor size.

6. The radiation dose at the skin is also difficult to interpret. Only 12 patients had it, and since this was a retrospective review, this measurements were not part of a protocol with informed consent.. I guess. How were those patients selected among the 90 patients? We do not know if they represent the entire cohort.

Reply 6: We obtained informed consent from all patients who were measured by wearable dosimeters. Actually, this was a prospective-retrospective study. The limited research grant allowed us to do it for only 12 patients at that moment. We needed 5 dosimeters per person, and total 60 dosimeters were used in this study. And, those dosimeters were available really in the latter half of this study.

7. Table 1 lacks some units (i.e. time, blood loss)

Reply 7: We added each units in Table 1.

8. Please spell check for a few typos.

Reply 8: Yes, spell check was done.

9. Figure 2b is supposed to show the clips on the surface of the patient and proximity to the lung lesion (per your text Line 138), but it only shows the lung lesion.

Reply 9: We replace it to another picture.

Reviewer D

Overall, this is an interesting and well-written manuscript assessing the utility of cone-beam computed tomography for the intraoperative localization of peripheral small pulmonary tumors. My comments are mostly minor.

1. Title. Given the preliminary nature of the analysis, I would suggest modifying the title to indicate that this is a "pilot study".

Reply 1: We corrected it, "A pilot Study of Intraoperative Localization of Peripheral Small Pulmonary Tumors by Cone-beam Computed Tomography: Sandwich Marking Technique".

2. Methods section. Selection bias always is a concern for small retrospective studies. Please assure the reader that all eligible patients were included and that none were arbitrary excluded.

Reply 2: We corrected the sentence "No patient was arbitrary excluded, but If a hybrid-OR is not available on the operation day, an alternative marking method such as preoperative percutaneous dye marking is used if a hybrid-OR is not available on the operation day (7)." (Page 7, Line 134-136).

3. Statistics section. P-values are needed to tell if the correlation coefficients are significantly different from zero.

Reply 3: All P-value was <0.0001 between MDCT and CBCT, between CBCT and Pathology, and between MDCT and Pathology. As you know, the fact means the correlation coefficients were not zero. We added "(P <0.001)" in Page 10, Line 223-225.

4. Line 40. "Tow" should be "two".

Reply 4: We corrected it (Page 3, Line 39).

5. Line 171. Please change "calculated" to "computed".

Reply 5: We corrected it (Page 9, Line 185).

6. Lines 176-177. Please justify 90% versus say 25, 50%, 75%, etc. How would the results changed if a different cutoff value was used?

Reply 6: The results shows same when a cutoff value would be 25%, 50%, 75%, or 90%. Because missing data was under 10% in all cases.