TRANSLATIONAL LUNG CANCER RESEARCH

Peer Review File

Article Information: Available at http://dx.doi.org/10.21037/tlcr-20-1231

Reviewer A

This is a retrospective, single-center analysis of 30 lesions (in 25 patients) of microwave ablation of lung nodules with ENB guidance. This included both lesions that had confirmed malignancy as well as those that were "radiologically suspicious".

Major:

1. I'm concerned that 83% of your cases didn't have a firm malignant diagnosis. 50% of nodules were negative for malignancy or no biopsy was done at all! How can you say you had 12-month control of something we don't even know is malignant? This needs to be very well clarified in your limitations section. You have proven the procedure is safe and mostly effective, but I don't think you can make claims of 12-month control when you don't even know if 83% of your cases were even cancer. The same goes for applying mRECIST criteria to something that isn't definitively cancer.

Author's Reply 1: We thank the reviewer for the comments. We agree that control rates cannot be determined from the majority of lung nodules which did not have histologically proven malignancies, with or without utilizing mRECIST criteria.

Changes in the text: We have highlighted the lack of histological proof of malignancy in the "Limitations" section (Line 352). We have removed the claim of "reasonable mid-term control rate" in the "conclusion" section of the abstract (Line 94). Instead, the major finding from our review is that bronchoscopic transbronchial microwave ablation is safe and efficacious.

Minor:

1. would recommend the use of the term bronchoscopic transbronchial microwave ablation. This is a more accurate descriptor of the procedure.

Author's Reply 2: Thank you for your great suggestion, which we agree is a more accurate term to describe our procedure, differentiating from the intrabronchial ablation techniques which have been previously described for intraluminal lesions.

Changes in the text: The recommended term "bronchoscopic transbronchial microwave ablation" has been applied throughout the text, including Title, Abstract and the Main Text.

2. In your introduction, you do not mention prior studies/abstracts with bronchoscopic transbronchial microwave ablation. This seems like it is highly relevant and should be included. There is an abstract from IASLC from several years ago with the same device and a more recent abstract from CHEST 2020 with another device.

Author's reply: Thank you very much for your informative updates. Both groups were pioneers in the field and their contributions to the field has been acknowledged in the present paper.
Changes in text: "More recently, small case series of image-guided transbronchial microwave ablation of lung tumours have been reported by 2 separate groups with favourable benefit-risk profiles" (Lines 301-303) with both IASLC and CHEST articles cited as Reference 30 and 31.
3. Why wasn't this prospective? A study of such nature should really be done under a dedicated prospective research protocol. Were they discussed at a multidisciplinary conference? Were they given the option to have the standard of care treatment (i.e., SBRT?). Did patients sign an informed consent statement?
Author's Reply 3: Thank you for the comments on these relevant and poignant issues. Our data represents the first-in-human series of transbronchial microwave ablation in our centre, and there was indeed a steep learning curve, for example, with regards to patient/lesion selection, equipment familiarization, cooperation with OT colleagues, etc. The series presented in this study act as a mini pilot study to prove the safety and technical feasibility of bronchoscopic transbronchial microwave ablation. We totally agree that a prospective study should be performed under a dedicated research protocol – in fact, we are conducting a prospective trial with results probably going to be available later in the year.
Changes in the text: All cases were discussed with oncologists in multi-disciplinary meetings, and patients were interviewed to discuss the option of SBRT, wedge resection, or bronchoscopic transbronchial microwave ablation. We obtained informed consent from all patients. These have been added to the "Trial Design" section (Lines 136-137).

4. EBUS was not performed on these patients. Staging was solely based on PET/CT imaging.
Author's Reply 4: Thank you for the comments. We have performed nodal staging for a few suspicious lymph nodes found on imaging, however the majority of cases only had small non-avid lymph nodes which did not warrant nodal staging.
Changes in the text: The above has been clarified in the section "Enrolment criteria" (Lines 147-149).
5. No information given on ablation time/power used. Only average total energy. This leaves out a lot of details.
Author's Reply 5: Thank you for your interest. We agree that this is important information.
Changes in the text: The average and range of ablation time and power used is updated in Table 2.
6. You said some biopsies were performed the day of the procedure. Was ROSE performed? I suspect not. But my concern again is that only 1 of 6 of these cases had malignancy actually confirmed.
Author's reply 6: We totally agree that ROSE would be more ideal, but this arrangement was limited by pathologists' manpower and unfortunately could not be arranged during the study

period. However, we did perform frozen section of biopsy on 6 of the cases. Despite only 1 had confirmed malignancy, we proceeded with microwave ablation of the lung nodules as all of them were radiologically suspicious and lack of histology confirmation could be due to sampling error of ENB biopsy. Also, for those cases, we obtained prior informed consent from the patients to proceed with ablation in case of no histologic confirmation. On the other hand, those patients who preferred to have confirmed diagnosis of malignancy before ablation were not included this analysis.

Changes in the text: The above has been clarified in the "Enrolment Criteria" section (Line 152).

7. You mention only the median number of CBCT spins. Authors need to list the total range of scans done.

Author's reply 7: Thank you for your suggestion. The total range of CBCT scans done is 4-12 for all cases.

Changes in the text: Please see further details updated in Table 2.

8. Was cross-country tool used in any case? If so, this needs to be described in detail in the methods section. How often was this used?

Author's reply: Cross-country transbronchial access tool was used in approximately half of our cases which did not have bronchus sign.

Changes in the text: The use of CrossCountry tool has been illustrated in Video 1A and 1B legends, in addition to steps 7 and 8 in E-table 1. The use of CrossCountry has also been added to the main text in "ENB microwave ablation procedure" section (Lines 163-168).

9. PTX occurred in 2 cases (but only 6.67%?). 2 out of 25 patients is 8%. (same for those that had post-ablation reaction). If you're doing this on a per lesion basis (which it would appear) you also need to report it based on per patient basis, especially for PTX. This also needs to be made very clear in table 3. See also line 305, this is very deceptive. You specifically say 2 patients…that's out of a total of 25 patients (not 30) therefore the percentage needs to be adjusted.

Author's reply: Thank you for your correction. Those percentages were on per-lesion basis as patients with 2 lesions were ablated in separate sessions on separate dates. We are sorry for the confusion caused.

Changes in the text: Table 3 has been updated to "per-lesion" basis as well as in the "Results" section and lines 312-313 (which was previously line 305).

10. What was overall cavity formation percentage after ablation?

Author's reply: At 1 month post-ablation, predominant cavity formation was found in 5 cases (16.7%) on CT scans. However, some smaller cavities/air bubbles were also formed within lesions with predominantly "GGO" post-ablation morphologies.

Changes in the text: Please see E-table 3.

11. Table 2: need to give ranges in addition to mean.

Author's Reply: Thank you for your suggestions.
Changes in the text: Table 2 has been updated to include ranges.
12. Should list average (and range) of number of ablations per lesion.
Author's Reply: Thank you for your suggestions. The average number of ablation per lesion is 1.13, and 1 to 2 ablations were needed per lesion.
Changes in the text: Table 2 has been updated to include the above.
13. Should list average (and range) of time and power (not just total energy delivered) for all ablations.
Author's reply: The average and range of time and power has been updated in Table 2.
Changes in the text: Table 2 has been updated to include the above.
14. Line 272: should say "bronchoscopic" not bronchoscopy.
Authors' reply: Thank you for the kind correction.
Changes in the text: "bronchoscopy" has been changed to "bronchoscopic" as per suggested.
15. Line 289, please specify "percutaneous"
Authors' reply: Thank you for the suggestion.
Changes in the text: "percutaneous" has been added to the text as suggested (currently line 295).

Reviewer B

The present manuscript reports on initial experience with electromagnetic navigation bronchoscopy-guided microwave ablation of lung nodules at a single center. This is a retrospective analysis reporting on technical feasibility and safety.

This manuscript would be an important contribution to the literature, providing one of the first clinical reports on microwave ablation via a bronchoscopic approach. As such, the manuscript is well suited to the journal. Some comments on aspects the authors should address are provided below.

- Introduction, indicate primary contraindications for surgery and anticipated fraction of patients with pre-malignant or early stage disease that would not be surgical candidates.
Author's reply: Thank you for your suggestions. Contraindications for surgery include prohibitive cardiorespiratory risks and inadequate lung function, while relative contraindications include extreme old age, frailty, likelihood of difficult one-lung ventilation due to previous contralateral major lung resection. In our experience, the fraction of patients with pre-malignant or early stage disease who turned out to be not surgical candidates is at the ~5-10% range, although the fraction can be as high as 28% in octogenarians according to studies. Therefore, we believe the target group of ENB microwave ablation include up to one-

fourth of patients diagnosed with early stage lung cancer especially in elderlies.

Changes in the text: The above has been added to the "Introduction" section (Lines 105-108)

- Study design/methods, section on ablation procedure, provide more information about the ablation system and catheter. The microwave frequency of operation, ablation catheter dimensions (diameter and length), and method of cooling (gas/water, temperature), if any, would be important to include. Indicate whether power levels noted in the text and tables refer to the generated power, or power delivered to tissue; if the former, provide an estimate of power delivered to the tissue.

Author's reply: Thank you for your interest. The microwave catheter used is Emprint™ Ablation Catheter with Thermosphere™ technology (Covidien™, Plymouth, MN, USA). The microwave output frequency is 2.45GHz +/- 5MHz, the active ablation tip is 1.8cm in length and 2mm in diameter. The system is water cooled. Power levels in the text and tables refer to the generated power. The power delivered to tissue depends on the tissue characteristics and environment, but unfortunately, we do not have the relevant information from the manufacturer. It may be an area for future research.

Changes in the text: The above information has been added to E-Table 1 footnotes.

- Results, table 1, provide ranges (in addition to provided means) for distance to fissure and distance to pleura data.

Author's reply: Thank you for your suggestion.

Changes in the text: Table 1 has been updated with ranges of distance to fissure and pleura as suggested.

- Fig 2B, provide contours to illustrate extent of the actual ablation boundary as assessed on CT imaging. In text (or table 2), specify how minimal and maximal margins were assessed. Difference of maximal linear dimension of the ablated region vs. tumor, or circumferential assessment of margin after overlaying tumor on ablation zone? Or perhaps some other method?

Author's reply: Thank you for your great suggestions. Contours have been added to Figure 2B and legends updated. Minimal and maximal margins were assessed circumferentially after overlaying tumour on ablation zone. The SuperDimension software allows for 3D appreciation of images so that the minimal distance between tumour border and ablation edge is defined as the minimal margin.

Changes in the text: Figure 2B has been updated to illustrate extent of actual ablation boundary. The method of measuring margins has been added to footnote in Table 2.

- If available, data on device positioning within the tumor (e.g. minimal/maximal radial distance to tumor boundary) would be informative to interpret the range of minimal/maximal margins reported in Table 2. Besides the tissue characteristics indicated, did geometric considerations (i.e. central vs. eccentric placement of catheter relative to tumor) have an impact on observed margin sizes?

Author's reply: Thank you for your insights. We reviewed our data and found out that the

ablation catheter was well within 17 out of 30 nodules, just adjacent to lesion border in 10, and away from lesion in 3 (although the ablation zone covers the lesion). In general, there is a higher chance of inadequate margin requiring second ablation if the catheter is just adjacent or not within the nodule. However, it also depends on tissue contraction, the size of lesion, etc. In our series, there is no statistically significant relationship between catheter position and eventual minimal margin.

Changes in the text: Information regarding catheter position in relation to the lesion is included in Table 2.

- Comment on appearance of ablation zone for re-ablation cases where ablated tissue would be in proximity to the catheter, and may have different response to applied energy compared to native tissue

Author's reply: From the several double ablation cases we have performed, we noticed no obvious difference in the appearance of re-ablated tissue after the second-time ablation. We speculate that the second ablation did not further change the overlapped region of ablation since the initially ablated tissue is desiccated and shrunken. An analogy is that completely dried food heats up minimally after microwave.

Changes in the text: nil

- Discussion, first paragraph, microwave heating is unlikely to be "completely independent" from electrical conductance and other tissue properties. This statement does not align with the last sentence of the 5th paragraph of the discussion. Consider revising to indicate microwave heating may be less dependent on electrical properties than RF heating, rather than completely independent. Computational modeling studies have shown that tissue electrical parameters have a substantial impact on microwave ablation zone dimensions.

Author's reply: Thank you for your kind suggestions and we agree with the change in wording.

Changes in the text: "Being less dependent on electrical conductance…" (Line 285)

- Recent reports on development and evaluation of new bronchoscopic ablation technologies using a range of energy modalities, in animal models should be acknowledged: Casal et al, J Bronchol Interv Pulmonol, 2018; Sebek et al, ERJ Open Res, 2020; Yoneda et al, 2020).

Author's reply: We totally agree that these pioneers in the field should be acknowledged in the present paper.

Changes in the text: "Lately, transbronchial ablation techniques using different energy source have been researched in animal lung models." (Lines 298-300) with Casal, Sebek and Yoneda's papers quoted as references 24-26.

- The comment on overestimation of ablation zone on CT imaging is appreciated, but extent of overestimation should be clarified. Meram et al, (J Vasc Interv Radiol, 29(10), 2018) report that both conventional and cone-beam CT imaging over-estimated ablation zone by 8-9 mm in lung.

Author's reply: Thank you for your astute input. Yamamoto et al (AJR Am J Roentgenol. 2005; 185(5)) found out that the overestimation is 4.1mm while Meram et al estimated overestimation

of 8-9mm. Both compared CT with pathological findings in porcine lung models. We totally agree that overestimation of CT ablation zone should definitely be taken into consideration when determining whether margin is sufficient or not.

Changes in text: Meram's study finding of 8-9mm overestimation is added to "Discussion" section (Line 327).

- The comment on 35 mm maximum ablation zone width should be augmented to indicate that these are from animal data (no tumor); currently that statement appears further down in the next paragraph.

Author's reply: We appreciate your kind suggestions and comments.

Changes in text: "For the Emprint microwave catheter used in the present study, the spheroidal maximal ablation zone width is 35mm at 100W for 10 minutes, being derived from in-vivo experiments in healthy porcine lungs." (Lines 329-330)

- Given the contrasting effects of tissue contraction (possibly leads to underestimation of ablation extent) and CT appearance of ablation zone (possibly leads to overestimation of ablation extent), the authors may comment on adequacy of existing imaging techniques for assessment of ablation zone, and their use for monitoring patient response to ablative treatment.

Author's reply: While we agree that existing imaging techniques for assessment of ablation zone (mainly by CT or CBCT) is only fairly reliable with reasons as mentioned above, we believe that it will remain the main modality for assessing and monitoring patient response due to its wide availability and low cost. Multi-phase CT, PET/CT and dynamic contrast-enhanced MRI have been used for monitoring of response to ablation with numerous advantages over CT, however they are limited by its availability and cost.

Changes in text: The lack of other imaging modalities for better assessment and monitoring of patient response to ablation has been added as a limitation to the present study (Lines 353-357).

- The images and videos of the procedure are informative, and methods to minimize movement of the bronchoscope and catheter during the procedure are appreciated. Were any data taken to assess catheter movement relative to target during the procedure? Would be informative to include.

Author's reply: Thank you for your kind comments. As a screening, fluoroscopy was obtained every 3 minutes during ablation to monitor any unexpected movement of catheter in relation to target lesion.

Changes in text: "Fluoroscopy obtained every 3 minutes to detect any inadvertent movement of catheter." added to E-Table 1.

Overall, this paper would be an important contribution to the literature after clarifying some of the items listed above.

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Reviewer C

Congratulations, for a retrospective paper one of the best I read in this area. It will be a cornerstone for papers in that area in future.

In detail:

Row 53: Radiologically suspicious iSPN should not be the goal for a prospective study as the overlap to benign nodules < 10mm is large. For a retrospective study, it is ok.
Author's reply: Thank you for your comments. We agree that for future prospective studies, only histologically confirmed malignancies in iSPN should be recruited.
Changes in the text: N/A

Row 55: A nodule size up to 29mm is critical despite the physical properties of the antenna. These nodules should be re ablated in any case - or treated simultaneously with EPR or ablate-refill-ablate on water-basis with cisplatin or paclitaxel. Look up my review.
Author's reply: Thank you for your insightful suggestions. We totally agree that re-ablation for larger tumours will likely improve tumour kill and margin, thus the three larger tumours (22, 26 and 29mm in diameter respectively) underwent planned double ablation in our series. Tumour targeting via EPR is also a promising field for personalized cancer treatment. A review on the combination of thermal ablation and chemotherapy delivered locally to a rabbit lung model also offers an exciting area of future developments.
Changes in the text: "Combined with simultaneous intra-tumoural chemotherapy(43), or enhanced permeability and retention (EPR) effect via hyperthermia induced by ablation(44), transbronchial lung cancer ablation will likely become an important part of the armamentarium in an exciting era of personalized cancer treatment" (Lines 361-363)

Row 56: A bronchus sign should be related to outcome in my opinion but future will tell us the truth.
Author's reply: We were also expecting bronchus sign to have a favourable impact on procedural time and ease of navigation, however there were none in our preliminary subgroup analysis. We speculate that it may be due to small sample size, and the fact that some lesions with positive bronchus sign required double ablation due to its larger size, making procedural time longer. The CrossCountry transbronchial access tool has also proven effective and easy to use in those without bronchus sign.
Changes in the text: nil

Rows 58: It would be highly interesting how you clearly made the difference between contraction effect and the predicted ablation volume difference -and especially at what point of time. Could you comment on this?

Author's reply: Thank you for your insightful queries. The contraction effect is measured on immediate post-ablation CT scan, at 10 minutes after ablation. The contraction percentage for a reference point at a particular distance from centre is calculated by (Pre-ablation distance – Post-ablation distance)/Pre-ablation distance x 100%. The reference points need to be identifiable in both pre- and post-ablation CTs, and are usually recognizable bifurcation of adjacent blood vessels.

The ablation volume difference between predicted and actual is also measured at 10 minutes after ablation. According to the ablation catheter manufacturer (E-Figure 6), the predicted ablation zone size is the experimental result from animal models, specifically, in in-vivo healthy porcine lungs, within minutes after ablation. This is compared to the actual ablation zone size in our patients. What we find is that the actual ablation zone size is on average smaller than that predicted (possibly due to differences in human lung texture, presence of hypervascular tumour, etc), but the variance in very big. In a few cases, the actual ablation size is much larger than predicted, and seem to be related to emphysematous lungs, although a statistically significant relationship cannot be demonstrated due to small sample size.

Changes in the text: Figure 3 legend is updated to include: "References points are usually recognizable bifurcations of adjacent blood vessels which can be identified in both pre- and post-ablation CTs."

Row 197: This is an essential information about mean total procedural time as those patients are very comorbid - and 126.3 minutes is much longer than 10 mins in SBRT! Therefore at the moment I still think that broncus sign (or equivalent) eases up the intervention - my personal goal is no longer 1h!

Author's reply: We totally concur that ablation time should be as quick as possible for patients with significant comorbidities, for example those who can hardly lie flat due to heart failure, and those who have high GA risk in the first place. A 10-minute SBRT might be simpler and safer in those cases. However, many of our patients simply were not surgical candidates due to inadequate lung function after previous major lung resection, and were thus able to tolerate bronchoscopic microwave ablation more. Nevertheless, we have been observing a general trend towards shorter procedural time as we progress along the learning curve, the shortest so far being less than an hour: roughly 5 minutes for ENB registration, 10 minutes for ENB navigation, 10 minutes for ablation, and a further 10 minutes for post-ablation CT scan.

Changes in the text: nil

Row 228: Very interesting that none of the typical factors for less than predicted ablation zone

size showed significant results.

Author's reply: We have been surprised by this fact too, but we suspect that this may be due to small sample size. In fact, localized heat-sink effects are still observed in some cases, for example a skewed ablation zone away from the side where a sizeable blood vessel is present. However, overall it did not reach statistical significance.

Changes in the text: nil

Row 263: Very interesting the information about shrinking variance.

Author's reply: Thank you very much for your kind comments.

Changes in the text: N/A

Row 350: I love the comments on lung densitometry and water content.

Author's reply: Thank you very much for your kind comments. We hope to find out the relationship between lung density/water content and ablation zone size in future studies.

Changes in the text: N/A