Author’s response to reviews

Title: The utility of transbronchial rebiopsy for peripheral pulmonary lesions in patients with advanced non-squamous non-small cell lung cancer

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Author’s response to reviews:

Responses to the Comments from the Editor and Reviewers

We thank the editor and reviewers for reviewing and critiquing our manuscript, as their valuable and insightful comments have helped to improve our manuscript and to highlight the significance of our research. We hope that our revised manuscript answers all your queries and is acceptable for publication in your journal. The changes and corrections made to the original manuscript are marked in yellow highlight.

Comments from Reviewer #1:

Reviewer 1: The authors present a manuscript of interest for interventional pulmonologists and those involved in lung cancer diagnosis. I recommend publish it. However, I have several comments for the authors that must be addressed:

ABSTRACT section:
Page 2, lines 3-4, background: The sentence "However, the differences….unclear" is unclear and does not match with to the objective of the manuscript. The aim of the study is to compare the diagnostic performance of rebiopsy with first biopsy both by means of R-EBUS. The sentence, as is written, means that the study compares the molecular profiles of the specimens when this is not the objective. It should be rewritten.
Page 2, lines 4-6: “However, the differences between rebiopsy and initial biopsy with regard to their diagnostic yields and their ability to test molecular profiles when using bronchoscopy with R-EBUS in patients with advanced NSCLC remain unclear.”

BACKGROUND section:
Page 4, lines 2-4: The sentence "differences in….explored". Again, is not a question of comparing molecular profiles but the ability to test molecular profiles.
→ Thank you for your comment. We have rewritten the sentence as below.
Page 4, lines 4-6: “however, the differences between rebiopsy and initial biopsy with regard to their diagnostic yields and their ability to test molecular profiles when using bronchoscopy with R-EBUS have not been explored”

METHODS section:
Page 5, line 2: The abbreviation GS (for guide sheath) is not included in the list of abbreviations. Please consider if necessary to use an abbreviation for words that are included only 3 or 4 times in the text, especially those that are not widely used.
→ Thank you for your comment.
Page 11, line 10: We have added the abbreviation of GS; guide sheath to the list of abbreviations.

RESULTS section:
Page 7, lines 8-10: Please, consider to describe the results of molecular profile analysis after comparing the results of diagnostic performance and before the complications rate between both groups. Since only EGFR was performed, I recommend to describe the EGFR testing instead of molecular profile performance.
→ Thank you for your comment. We have changed the order of the sentences. Page 7, lines 17-22: We have described the results of molecular profile analysis after comparing the results of diagnostic performance and before the complications rate between both groups.

Page 7, lines 11-16: In my opinion, this paragraph is not necessary and does not add relevant information to the study. The differences of molecular profile between both groups do not depend on the diagnostic performance of both techniques. In a different way it would be interesting to describe the rates of successful molecular testing between both groups but not the results of these tests.
→ Thank you for your comment. We have deleted the results of T790M testing, ALK rearrangements analysis and PD-L1 TPS, instead describing the success rate of ALK rearrangements analysis.
Page 7, lines 17-22: “Molecular profiles were evaluated in a proportion of cases (71 in the rebiopsy group and 134 in the initial biopsy group for EGFR mutation analysis, 8 in the rebiopsy group and 123 in the initial biopsy group for ALK rearrangements analysis). Adequate tumour samples were obtained from 93.0% (66/71) rebiopsy and 94.0% (126/134) initial biopsy patients for EGFR mutation analysis (p=0.765), and from 100% (8/8) rebiopsy and 99.1% (122/123) initial biopsy patients for ALK rearrangements analysis (p=1.000).”
DISCUSSION section:
Page 7, line 19. I think the first sentence of the discussion is not accurate or does not describe the aim of the study properly. The aim of the study was to compare the diagnostic yield of R-EBUS-guided TBB for PPLS in both first biopsy and rebiopsy after treatment. Please consider rewrite the sentence
→ Thank you for your comment. We have rewritten the sentence as below.
Page 8, lines 2-3: “We conducted this study to compare the diagnostic yield of R-EBUS-guided TBB for PPLs between initial biopsy and rebiopsy after treatment.”

→ Thank you for your comment.
Page 8, line 3-4: We have rewritten the sentence as your suggestion.

Page 8, line 9: Previous studies revealed that (tumor) size
→ Thank you for your comment.
Page 8, line 23: We have added “tumor” in front of “size”.

Page 9, lines 2-3. The sentence "Additionally...group" is unclear. Please rewrite. Do they refer to the use of guide sheath? How do you measured this in percentages? Is it relevant or can have clinical impact?
→ Thank you for your comment. We have referred to the use of a guide sheath in the Methods section (page 5, line 8) and have recorded all procedures wherein it may be used. However, we have deleted this sentence as it is not necessary for this article.

Page 9, line 11: The end of the sentence is unclear, consider rewrite it.
→ Thank you for your comment. We have rewritten the sentence as below.
Page 9, lines 24-25, Page 10, line 1: “Additionally, a rebiopsy is necessary not only for EGFR mutation-positive NSCLC to detect T790M, but also for analysing ALK rearrangement, PD-L1 TPS, and aberrations in BRAF, and ROS1, and to detect other genes including using NGS.”

OTHER COMMENTS:
There are two other comments that I would like to make and, in my opinion are important:

1) The authors describe the diagnostic yield of the technique in two different settings. However, as the authors affirm, although it is important to establish a diagnosis, in the rebiopsy setting is more important to obtain samples that allow molecular profiling. I recommend to include the success ration of molecular profiling between both groups. The authors only describe the results of successful sampling with the EGFR testing. Then, present differences of T790M mutation, ALK and PD-L1 testing in the samples but don't give the results of successful testing of these markers in both groups.
→ Thank you for your comment. Page 7, lines 17-22: We have described the success rate of molecular profiling between both groups, instead have deleted T790M mutation and ALK rearrangement.
2) Probably the best way to compare the diagnostic yield of the technique in both different clinical scenarios would be to compare each of the different diagnostic settings prospectively in the same patient. However, the study is retrospective, and, as the authors affirm, has this limitation. Nevertheless, probably some of the patients included in the rebiopsy group were also included in the first biopsy group. It would be interesting to see the results of both techniques in this group of patients.

→ Thank you for your comment. We agree that the best way to compare the diagnostic yield is to compare diagnostic performance in a prospective setting. Comparing the diagnostic outcomes of rebiopsy and initial biopsy of the same patients would be a good idea. However, during our study period, patients who underwent both initial biopsy and rebiopsy for PPLs were very few. We would certainly consider comparing the diagnostic outcomes for such cases in the future.

Comments from Reviewer #2:
Reviewer 2: Original article that attempts to compare 1) the diagnostic yield and molecular profile of the rebiopsy specimens compared to the initial biopsy specimens, and 2) the factors affecting diagnostic yield of rEBUS-guided TBBs for PPLs before and after treatment; in 301 patients with advanced non-Sq NSCLC.

I find the objectives of the study very interesting and original. However, there are a few issues I would like to address.

First of all, the authors use the terms "diagnostic data" and "features" throughout the text without clarifying at any point what these refer to. PPL characteristics (eg. tumor size, location, visibility on chest X-ray…)? Viability of the tumor cells in the specimen? The molecular profile of the lesions? I think that clarification from the beginning would ease the efforts needed to follow the progress of the objectives throughout the text.

→ Thank you very much for your insightful suggestion. According to your advice, we have replaced the terms “diagnostic data” and “features” with more specific terms as shown below.

Page 2, line 6: “diagnostic data” → “diagnostic yields and ability for molecular analyses”
Page 4, line 7: “features” →” the diagnostic yields and ability for molecular analyses”
Page 4, line 21 / Table1: “features” → “morphology (solid or part-solid)”

*Background:
- The sentence "to compare the features of patients" [...] is too vague. In the last paragraph of this section the objectives of the study should be clearly defined and developed accordingly throughout the text.

→ Thank you for your comment. We have written the sentence as below.
Page 4, lines 7-9: “to compare the diagnostic yields obtained from rebiopsy to those obtained from initial biopsy (first diagnosis) and their ability to test molecular profiles by means of bronchoscopy with R-EBUS”

*Methods
- In the patient selection paragraph, the term "features" is confusing. From table 1 I deduced that it refers to nodule type (solid or part-solid). Please clarify.

→ Thank you for your comment.
Page 4, line 21: We have replaced the term from “features” to “morphology (solid or part-solid)”.

- Regarding the paragraph on bronchoscopy procedures:
  - Ziostation is NOT a virtual bronchoscopy navigation (VBN) platform. Instead, it is a workstation were virtual bronchoscopy (VB) can be performed. I encourage the authors to change the terminology in order to avoid confusion, although I find the explanation provided is acceptable.
    → Thank you for your comment. According to your advice, we have rewritten the following sentence to avoid confusion.

Page 5, lines 9-10: “Virtual bronchoscopy constructed using a workstation (Ziostation2; Ziosoft Ltd., Tokyo, Japan) was prepared”

- In lines 6-7 pg 5, the authors say that "A PPL was defined as an abnormal growth […] that was bronchoscopically invisible". I suggest defining a PPL as a lesion not accessible with a conventional bronchoscope. Moreover, the authors should specify the conventional bronchoscope they used (model and perhaps also inner/outer diameters).
  → Thank you for your comment. We have rewritten the sentence as below.

Page 5, line 13: “ A PPL was defined as an abnormal growth […] that was not accessible with a conventional bronchoscope.” In addition, we have specified the conventional bronchoscope that we have used.

Page 5, lines 4-7: “TBB procedures were carried out using any one of the following conventional flexible bronchoscopes (Olympus Ltd.): BF-1T260, BF-260, BFP260, BF-F260, BF-1TQ290, BF-Q290, BF-P290, LF-TP, or BF-Y0053.21

- The term "target bronchus" should be defined. Is it the afferent? The one leading to the PPL?
  → Thank you for your comment. We have rewritten the sentence as below.

Page 5, lines 13-14: “Upon reaching the target bronchus which leads to the PPL, the R-EBUS […]”

- Most of the PPLs were visible on chest RX (CRX). However, a few were not. In those cases with CRX-invisible PPLs, did the authors use fluoroscopy too?
  → We used fluoroscopy in all cases regardless of whether the PPLs were CRX-visible or invisible. We have added the following sentence.

Page 5, lines 18-20: “X-ray Fluoroscopy (VersiFlex VISTA, Hitachi, Japan) was used in all the cases for guiding the insertion of the R-EBUS probe regardless of PPL visibility on chest radiography.”

- How many TBBs were performed? Did the number of TBBs taken differ between groups? I finally found this information in the discussion BUT it should be specified in the methods section.
  → Thank you for your comment. We routinely counted the number of TBB specimens in all cases. We have added the following sentence to the Methods as well as the Result section.

Page 5, lines 21-22: “Five or more specimens were collected from the patients as possible. The number of TBB specimens was counted in all cases.”
Page 6, lines 21-23: “At least three specimens were taken from all patients. Five or more specimens were collected from 89.9% of the patients in the rebiopsy group and from 88.3% of those in the initial biopsy group in the last analyses.”

- Please explain how ROSE of the TBB specimens was performed. Also, if the result of the ROSE determined any changes during the procedure (e.g. changing the site of biopsy). Maybe the TBBs in the rebiopsy group had smaller amounts of viable tumor cells and a greater amount of biopsies were needed?
  → Thank you for your comment. The final diagnosis of ROSE would not be made in the examination room because of the absence of pathologists there, and so that our procedure process would not be influenced by ROSE results. There was no evidence of improvement in diagnostic yield using ROSE. In addition, we took five or more specimens as possible, and the proportion was almost the same in both groups (89.9% vs. 88.3%). We have deleted the sentence about ROSE to avoid confusion.

*Results:
- I suggest simplifying the flowchart of the study (figure 1) since there are some confusing points as what "failure" or "success" are, or what "due to clinical trial" means. Otherwise, specify in the figure footnote.
  → Thank you for your comment. We have added the figure footnote of “failure”, “success”, and “due to clinical trial”.

- The authors forgot to specify in the text that also distance from costal pleural differed significantly between groups (p=0.031).
  → Thank you for your comment. We have written the sentence as follows.
Page 7, line 3-4: “There were significant differences in the median ages of patients in the rebiopsy and initial biopsy groups (64 [range, 57–69] years vs. 68 [range, 59–75] years, p=0.001) as well as sex (males, 38.7% vs. 53.8%, p=0.012) and distance from costal pleura (mm) (≥ 10.0, 44.3% vs. 31.8%, p=0.031).”

- In the results table, I would include the diagnostic yield (not only the p value) as well as the rates of detection with the rEBUS for each group.
  → Thank you for your comment. We have added the table of diagnostic yield and R-EBUS detection in each group (Table2).

- I find Table 2 not self-explanatory. I recommend changing the titles "univariate" and "multivariate" in order to simplify the lecture and understanding of the results.
  → Thank you for your comment. We have added a row of percentage of each variables and changed the titles of Table 3.

- Please specify what "moderate bleeding" refers to.
  → Page 7, line 23: We have added the definition of moderate bleeding. “(defined as 25-100 ml of blood)”.
Please specify why "molecular profiles" were evaluated in only a proportion of cases since the main objective of the study was to compare the diagnostic yield and molecular profile of both groups. Also, I would find interesting to add the molecular profiles of each group in a table.

→ Thank you for your comment. First, the patients in this study were a heterogeneous group in the clinical practice. Those who underwent molecular analyses were patients with successful pathological diagnosis. In addition, among the patients with successful pathological diagnosis, those who underwent biopsy for the registration of clinical trials, and whose doctors’ decided not to analyse the molecular profiles were excluded. We have rewritten the main object of the background section, and added the success rate of molecular analysis to the results section as below to avoid confusion.

Page 4, lines 7-9: “to compare the diagnostic yields obtained from rebiopsy to those obtained from initial biopsy (first diagnosis) and their ability to test molecular profiles by means of bronchoscopy with R-EBUS”

Page 7 lines 17-22: “Molecular profiles were evaluated in a proportion of cases (71 in the rebiopsy group and 134 in the initial biopsy group for EGFR mutation analysis, 8 in the rebiopsy group and 123 in the initial biopsy group for ALK rearrangements analysis). Adequate tumour samples were obtained from 93.0% (66/71) rebiopsy and 94.0% (126/134) initial biopsy patients for EGFR mutation analysis (p=0.765), and from 100% (8/8) rebiopsy and 99.1% (122/123) initial biopsy patients for ALK rearrangements analysis (p=1.000).”

*Discussion
- The authors start the discussion pointing out a vague sentence on the objectives of the study. Instead, the main results should be pointed out, one by one, at the beginning of the discussion.

→ Thank you very much for your suggestion. According to your advice, we have added the following sentences to the beginning of the discussion section.

Page 8, lines 5-8: “In addition, on multivariate analysis, factors affecting the diagnostic yield of TBB were positive bronchus sign and for those located in the internal two-thirds of the lungs. Our data revealed that rebiopsy did not significantly affect the diagnostic yield.”

- In line 1 p.8 the authors state that the fact that they used rEBUS together with a navigation system may underlie their satisfactory diagnostic yield. Again, I disagree with the authors when they say that Ziostation is a navigation system. Moreover, it has been previously demonstrated in a RCT that VBN combined with rEBUS increases the diagnostic yield for PPLs (Ishida T, Asano F, Yamazaki K, et al. Virtual bronchoscopic navigation combined with endobronchial ultrasound to diagnose small peripheral pulmonary lesions: a randomised trial. Thorax. 2011;66(12):1072-1077. doi:10.1136/thx.2010.145490). However, again, I think that the so-called navigation in this study is not comparable to the VBN platform used in the aforementioned study.

→ Thank you very much for your comment. We have rewritten as below.

Page 8, line 12-16: “We believe the high within-rate of R-EBUS detection contributed to our satisfactory diagnostic yield. The particularly large sizes of the tumours observed in advanced lung cancer, the high rate of positive bronchus sign (which are associated with tumour size), and the accurate guidance provided by some kinds of navigation (achieved using a workstation), might underlie the good result of R-EBUS detection.”
In line 12 p. 8 the authors introduce the term "repeat biopsy". However, the patients were not the same in both groups and therefore I would keep using the term "rebiopsy" throughout the text.

→ Thank you very much for your suggestion.
Page 9, line 1: We have changed the term “repeat biopsy” to “rebiopsy”.

- In the same paragraph, the authors state that "while rebiopsies are considered more difficult […] our results are not surprising because we used rEBUS for all patients together with GS". Please explain why using rEBUS-GS would be better and make proper citations when appropriate.
→ We have added the sentence below.
Page 9, lines 6-7: “Wedging the GS in the target bronchus might help stopping the bleeding after TBB.21”

- In the same paragraph, explain the 30 mm cut-off. I do not understand.
→ The enrolled patients in this study had advanced lung cancer. We decided to use 30 mm as a cut-off because the tumours sizes were mostly larger than the 20 mm cut-off which is usually used. We have rewritten the sentence as follows.
Page 9, lines7-8: “Moreover, we used 30 mm as a tumour size cut-off as the majority of the tumours were larger than the upper limit of T1 factor; the median length was 38 mm.”

*Conclusions
- Misspelling of "molecular" in the last sentence of the conclusions (both in the abstract and in the text).
→ Thank you for your comment. We rewrote that misspelling.
Page 10, lines 20: “moleculer” → “molecular”

Once again, we would like to thank Dr. Ernest Nadal (Editor-in-Chief), reviewer 1, and reviewer 2 for their dedicated and insightful review of our manuscript and the consideration of our manuscript for publication in BMC Pulmonary Medicine. I look forward to hearing from you.

Sincerely,

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