Peer Review File
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Reviewer A

Comment 1: T**he term "re-biopsy" is generally used for the cases performed after the second time biopsy following induction treatments. The authors meant that "repeated biopsy (re-biopsy)" was the second time biopsy after negative result by EBUS-TBNA. It should be clearly explained in the manuscript to avoid misleading the reader.**

Reply 1: Thank you a lot for point out this mistake, we do confuse the definition of "Re-biopsy". This statement in whole manuscript and title was changed by "repeat-biopsy".
Change in the text: The statement of "re-biopsy" was replaced by "repeat-biopsy" in the revised manuscript.

Comment 2: Reference no.3 was inappropriate because reference no.3 was the article that was written about the EBUS guide sheath procedure for peripheral pulmonary lesions.

Reply 2: It is really true as Reviewer suggested that we used a wrong reference, we carefully chose a suitable reference and re-written this sentence
Change in the text: In the first paragraph, the statement of " So far, some studies have reported failure of EBUS-TBNA in the diagnosis of mediastinal lymph nodes and peribronchial lesions. In such cases, repeat biopsy under EBUS guidance (re-biopsy) is generally performed, although there is no consensus regarding the efficacy of re-biopsy" was re-written as " So far, some studies have reported that EBUS-TBNA may not precisely diagnose mediastinal lymph nodes and peribronchial lesions for some situations. Therefore, to avoid a repeat sampling procedure, a better method of biopsy should be applied. However, given the high sensitivity and specificity of EBUS-TBNA method, repeat EBUS-TBNA (repeat-biopsy) may yield satisfactory results."
The reference no.3 "Factors leading to failure to diagnose pulmonary malignant tumors using endobronchial ultrasound with guide sheath within the target lesion" was replaced by "The diagnosis of lung cancer in the era of interventional pulmonology."

Comment 3: What did the "atypical results" mean? Did the authors suggest "atypical cells" by cytopathology?

Reply 3: Thank you very much for pointing out this misleading description. "Atypical result" was defined as EBUS-TBNA result which has abnormal cytopathology results but has no diagnostic value. We have re-written this part in the article to clearly explained this point.
Change in the text: At line 39, the statement of "55 patients had atypical results with

abnormal pathological examination results which were not confirmed;" was re-written as " 55 patients showed atypical results, i.e., abnormal cytopathology results with no diagnostic significance;"

Comment 4: Even for the negative results, why did the authors perform repeated EBUS-TBNA for this population? I think the authors did not perform repeated EBUS-TBNA routinely for all patients.

Reply 4: Thanks for the reviewer's comment. From 4911 patients who underwent EBUS-TBNA procedure, we screened out 140 patients who receive repeated EBUS-TBNA biopsy. As you can see, this is a very small part of this population. **All patients received multiple examinations and had a clear indication of EBUS-TBNA. So, when the results of EBUS-TBNA were not consistent with what was expected, the repeated biopsy may be contemplated.** And, We do gain different cytopathology or pathology result from the repeat-biopsy in some patients. However, there were still some patients can't benefit from repeat-biopsy (like the patient we mentioned in the manuscript). We conducted this retrospective analysis to explore the efficacy of repeat-biopsy.

Comment 5: Did the authors use rapid on-site evaluation during EBUS-TBNA?

Reply 5: Due to the big time span of this retrospective analysis(from January 2012 to June 2020), only a part of patients received ROSE evaluation. About the performance of ROSE in EBUS-TBNA, we discussed this point in the penultimate paragraph.
Change in the text: We have re-written the last paragraph, and the statement of "Rapid on-site evaluation (ROSE) is a simple procedure that cam provide an immediate and accurate assessment of benign and malignant samples. Moreover, the procedure can avoid additional sampling. However, there is no consensus on the necessity of ROSE in combination with EBUS-TBNA.." was add at line 90.

Comment 6: It was unclear the mean of "the region from which biopsy is taken." Did the authors take the biopsy from different stations?

Reply 6: We are sorry for our misleading statement, which will be re-written in the revised manuscript. EBUS-TBNA was a novel technique for mediastinal lesions, so we separate the mediastinal lesions into two different groups, mediastinal lymph nodes, and peribronchial lesions, trying to figure out whether the biopsy site affects the correct rate.
Change in the text: Line 55, "The region from which biopsy is taken" was correct as "It was found that site of biopsy may greatly affect the correct rate.".

Comment 7: The nodal staging should be the systematic sampling at least from more than three stations.

Reply 7: As the reviewer suggested, all EBUS-TBNA of lymph nodes was based on the CT or PET/CT scan imaging data, and at least three stations of target lymph nodes were sampled. As above mentioned that this retrospective analysis included EBUS-TBNA of mediastinal lymph nodes and peribronchial lesion, so we didn't describe the detail of lymph nodes biopsy in the manuscript.

Comment 8: Following the several current guidelines, the negative results by needle biopsy procedure were warranted to perform the surgical biopsy. This was a retrospective review, and why did the authors perform repeated needle biopsy procedures for these cases?

Reply 8: Thanks for this comment. As you said that, this is not a randomized control trial, we did not ask the patients for receiving repeat-biopsy. When the EBUS-TBNA gains an unexpected result, multiple options could be chosen, like endobronchial brushing, mediastinoscopic biopsy, and so on. If the bronchoscopist and physician considered that it is necessary to repeat EBUS-TBNA or patients refused the other biopsy modality, the repeated biopsy was performed on these cases. We screened this population from 4911 patients and hoped to figure out the efficacy of repeat-biopsy from this retrospective analysis.

Comment 9: Due to the above reason, the patient background might be affected by bias. Hence. It could not conclude the efficacy of repeated EBUS-TBNA by the authors' analysis.

Reply 9: Special thanks to you for your constructive comments. We have adopted a variety of strategies to avoid bias. Multiple clinicians from the pneumology department and the thoracic surgery department are involved in the decision-make of EBUS-TBNA, which included the decision of repeat-biopsy. On the other hand, the procedures of repeat-biopsy were conducted by different bronchoscopists if possible. We hope our answers to your comments may change your opinion about this article. Thanks again for your good advice.

Reviewer B

Comment 1: Was EBUS intranodal forceps biopsy performed in any patient during repeat procedures?

Reply 1: Thank you for this excellent comment. We conducted EBUS-INF procedure in some patients. But, we did not test its performance during the repeat procedures. EBUS-INF truly have the potential to be an effective biopsy modality for repeated EBUS biopsy.

Comment 2: Please discuss the utility of intranodal forceps biopsy and intranodal

cryobiopsy as these may be useful for repeat procedures.

Reply 2: As Reviewer suggested that we add this point in the discussion part.
Change in the text: The statement of "Recently, EBUS-guided intranodal forceps(EBUS-INF) biopsy has been proved as a novel technique that can provide larger histologic tissue sample. Using sample from EBUS-INF to perform genetic analysis may avoid repeat-biopys for insufficient tissue sample. Meanwhile, for suspected false-negative EBUS-TBNA result, EBUS-INF can be an alternative method for confirmation purpose" was add at line 90.

Comment 3: The authors need to clearly describe how to select patients for a repeat procedure

Reply 3: It is really true as the Reviewer suggested that we should clearly describe this point in the manuscript.
Change in the text: At line 38-45, the statement of "The 140 patients were divided into 4 groups according to reasons for re-biopsy: 1) 55 patients had atypical results with abnormal pathological examination results which were not confirmed; 2) 24 patients had negative results; 3) 17 patients underwent repeat biopsy due to poor quality of samples; 4) 44 patients received multiple procedures to review the disease or for genetic testing. In the following section, we discuss the first three group of patients." was rewritten as "Except 44 patients show received multiple procedures with the aim of reviewing disease or genetic testing. 96 patients received repeat biopsy due to unexpected or non-diagnostic results in the first biopsy. These patients were further divided into 3 groups based on reasons for the repeat-biopsy: 1) 55 patients showed atypical results, i.e., abnormal cytopathology results with no diagnostic significance; 2) 24 patients had negative results; 3) 17 patients underwent repeat biopsy due to poor quality of samples.".