Peer Review File

Reviewer A

This is a well-written manuscript where the authors have reported an association between cigarette smoking, pSUVmax and EGFR mutation status. These variables are interconnected in the context of non-small cell lung cancer, but the relationships are complex and not very straightforward to explain.

Reply: Thank you very much for your positive and objective comments on our manuscript. We have attempted to illustrate the associations between cigarette smoking, pSUVmax and EGFR mutation status more clearly in our study.

Cigarette smoking is one of the best-established risk factors for the development of lung cancer, and the risk is directly related to the duration and intensity of smoking. Smoking is more associated with squamous and small-cell lung cancer whereas non-smokers tend to develop adenocarcinoma histology. The authors have not made a histology-related comparison with respect to smoking and pSUVmax.

Reply: Thanks very much for your suggestions. Yes, studies have reported that smoking is more strongly associated with squamous and small-cell lung cancer, while non-smokers tend to develop adenocarcinoma histology. However, due to the limited number of patients with squamous cell lung cancer, we did not further evaluate the comparison between histology-related factors and smoking in terms of pSUVmax.

Changes in the text: None.

pSUVmax measures tumor metabolic activity and high pSUVmax values on PET scans are associated with more aggressive tumors, regardless of EGFR mutation status or smoking history. It can be an indicator of the tumor's metabolic activity and may influence treatment decisions. The AUC value lies between 0.6 and 0.7, which implies acceptable or moderate discriminative potential. The authors need to provide more proof of the evidence that the associations they have found are not chance-driven.

Reply: Thank you very much for your suggestion. The patients included in our study were continuously and randomly assigned. It is not based on chance.

Changes in the text: None

Also, EGFR-mutated NSCLC tumors are more likely to respond to EGFR TKIs, regardless of smoking history or pSUVmax values. In contrast, non-EGFR-mutated tumors, which are often associated with heavy smoking, may harbor many genomic alterations acting together. Could it be the reason for a high pSUVmax the authors are observing in non-EGFR mutated tumors? Such tumors generally require different treatment strategies, such as chemotherapy or immunotherapy. The authors need to make these points clear in the discussion section.

Reply: Thank you for your suggestion. Indeed, EGFR-mutated NSCLC tumors are more likely to respond to EGFR TKIs; however, their response may be influenced by factors such as

cigarette smoking history or metabolic activity, e.g., pSUVmax. Heavy smoking may result in non-EGFR mutated tumors and a high pSUVmax. We have made a discussion about this phenomenon in page 10, lines 290-295.

EGFR mutations are more prevalent in East Asian ethnicities and in women. The authors report a 46% EGFR mutation rate. Were the cases tested randomly or were they based on the physician's discretion? Also, it is not clear which method was used for detecting the EGFR mutation. Was it single-gene testing, which has a limited capacity for detecting EGFR variants, or an NGS-based method that can detect rare variants too? Please mention the method of mutation detection in the methods section.

Reply: Thank you very much for your suggestions. The cases that were tested for EGFR mutations were determined by the physician's discretion. We obtained the testing results, and patients who met our criteria were included in the study. We added the methods of testing EGFR mutations from our previous study, please see ref.27 in page 5, line 129.

The authors can mention whether the detailed smoking history was file-based or whether the patients/ their guardians were contacted to fetch the information.
The study is limited by a lack of patient treatment, prognosis and survival analysis.
In the conclusion section, the word 'obviously' may be replaced by some other phrase.

Reply: Thank you very much for your suggestions. In our study, all patients underwent a face-to-face interview prior to the PET/CT scan, during which the detailed information regarding their smoking history was obtained. We added this information in page 6 lines 166-167. Yes, one limitation of the study is the lack of analysis on patient treatment, prognosis, and survival, as noted on page 10 lines 310-313. In the conclusion section, we have replaced "obviously" with "notably."

Overall, the comments and suggestions you provided are truly valuable, and would like to express my sincere appreciation for them. Thank you.

Reviewer B

The study unveils intriguing findings regarding the association between metabolic phenotypes and EGFR mutation status in non-small cell lung cancer (NSCLC) patients. Notably, it reveals that EGFR-mutant NSCLC exhibits a lower pSUVmax compared to their EGFR wild-type counterparts (8.9 ± 4.5 vs. 12.7 ± 6.9, P<0.001). Additionally, smokers display a higher pSUVmax in comparison to never-smokers (12.5 ± 6.4 vs. 9.9 ± 5.9, P=0.004), with a noteworthy positive correlation observed between pSUVmax and cumulative smoking dose (r=0.198, P=0.005). Importantly, no significant differences were observed between nSUVmax and mSUVmax in patients, regardless of their EGFR mutation status and smoking history.

Reply: Thank you very much for your comments.

1. The study prompts a thought-provoking question regarding the variation in SUVmax values across primary tumors, lymph nodes, and metastatic tumors. It is crucial to explore the reasons

behind this phenomenon, especially considering that the specimens used for EGFR mutation analysis were obtained exclusively from primary tumors.

Reply: Thank you very much for your suggestions. Yes, it is crucial to comprehend the underlying reasons why pSUVmax exhibits significantly higher levels in non-EGFR mutant NSCLC compared to EGFR mutant NSCLC, while no significant difference was observed in nSUVmax and mSUVmax between them. However, further prospective investigation is warranted in future analyses, particularly through a point-to-point evaluation comparing SUVmax and EGFR mutation status. This could involve testing the EGFR results obtained from primary tumors, lymph nodes, or metastatic sites alongside corresponding SUVmax values derived from the same anatomical regions.

Changes in the text: None.

2. It is well-established that lung cancer patients with a history of smoking and elevated pSUVmax values generally experience lower survival rates when compared to their non-smoking counterparts with lower pSUVmax levels. Furthermore, it has been previously reported that patients lacking a smoking history tend to exhibit a significantly lower SUVmax in their primary and metastatic tumors, which is often associated with EGFR mutation status. However, it is imperative to acknowledge that in the current landscape of rapid EGFR mutation testing, the use of PET SUVmax or smoking status as predictive tools may no longer provide novel clinical insights. Therefore, the study, while informative, may not contribute significantly to the existing body of clinical knowledge in this era of advanced EGFR mutation testing methods.

Reply: Thank you very much for your comments. Albeit rapid EGFR mutation testing is available in some areas, the majority of the results are still based on tissue-based analysis. In this study, predicting the EGFR mutation status is a part of the investigation. The correlation between pSUVmax, smoking history, and the status of EGFR mutations is also important because it might reflect treatment response and prognosis.

Changes in the text: None.

1. I congratulate the authors for their work. I think that the Mann-Whitney u test should not be used in line 180 in the statistical analysis section. Mann Whitney u test is used to evaluate non-parametric data of two non-normally distributed groups in small sample sizes. Since the current study does not have a small sample group (n>30), it would be safer to use the Student't test. It is not stated whether the data is normally distributed or not.

Reply: Thank you very much for your valuable comments. In this study, the PET/CT parameters, namely pSUVmax, nSUVmax, and mSUVmax, were compared between patients with or without EGFR mutations using the Mann-Whitney test. Due to the abnormal distribution of the PET/CT parameter values (data not shown), we employed the Mann Whitney U test for our data analysis. Thank you again for your suggestions.

Changes in the text: None.

2.The evaluation of cumulative smoking dose and metabolic phenotype together in the ROC curve is well demonstrated. However, I think figure 3 is not very necessary.

Reply: Thank you very much for your valuable comments. Actually, although the evaluation of cumulative smoking dose and metabolic phenotype together in the ROC curve is well demonstrated, it would be more specific and graphic to provide an example to illustrate the correlation between them. Therefore, we included representative PET/CT images.

Changes in the text: None.

3.In the discussion section (232-255 lines), the findings of the study are repeated at great length. Briefly, it would be appropriate to state the most important of the findings in the first paragraph and then discuss them in the light of the literature.

Reply: Thank you very much for your suggestions. We have removed certain words and rephrased the sentences in the first paragraph. Please refer to page 9, lines 250-252.

4.When we look at the conclusion of the study, it does not provide a primary contribution to clinicians. The fact that EGFR mutation was not evaluated in all patients is an important limitation. However, metabolic activity, EGFR mutation and smoking can be evaluated together and can contribute to prognosis. However, clinicians will continue to need tissue diagnosis to plan treatment. I recommend that the contribution to the clinician section be detailed.

Reply: Thank you very much for your comments and suggestions. One limitation is that not all patients underwent testing for EGFR mutation status, which we have mentioned in the discussion section on page 10, lines 297-299. Indeed, our study presents novel information to clinicians by elucidating the associations among cigarette smoking history, metabolic phenotypes, and EGFR mutation status in patients diagnosed with non-small cell lung cancer. Our findings provide insights into the potential impact of cigarette smoking and pSUVmax on the development and progression of NSCLC, which may have implications for personalized targeted EGFR therapies.

Changes in the text: None.