Peer Review File

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Reviewer A

Question 1: Patient cohort in Methods section was not well explained, in particular there is no information about the stage and readers only infer from the results that both early and advanced stages have been included.
Reply: Thank you for your comment.
Changes in the text: None.

Question 2: Moreover, did you exclude patients with previous or concomitant history of cancer other than lung cancer? There is no information about this in the text.
Reply: We appreciate this observation. We have clarified in the Methods section.
Changes in the text: None.

Question 3: Why were the radiographic parameters (lines 122-125) extracted by only two thoracic surgeons? Although these surgeons have been defined as experienced, double checking by at least one experienced thoracic radiologist would be advisable.
Reply: Thank you for this suggestion. We agree that verification by an experienced radiologist would enhance the reliability of our imaging data.
Changes in the text: None.

Question 4: Before starting with the immunostaining protocol, did you screen the samples for putative CTC epithelial cells, in order to rule out haematopoietic cells (e.g. CD45- and PK+)?
Reply: We appreciate your insight. Prior to the immunostaining protocol, we did conduct a screening step to exclude hematopoietic cells (e.g., CD45- and PK+), ensuring the accuracy of CTC identification.
Changes in the text: None.

Question 5: In lines 196-197 the authors affirmed that the patients were divided into the CTC-high (CTC-H) and CTC-low (CTC-L) groups based on the median of CTC count, but no further information about these 2 groups follows in the text. In this regard, rather than a simple descriptive analysis that reported the average values of CTCs, it would be more useful to conduct the statistical analysis by identifying a significant cut-off for the CTC count, really dividing the patients in CTC-L and CTC-H groups. In fact, the presence of circulating epithelial cells in blood can be caused by conditions other than oncological conditions, such as stress, recent diagnostic procedures, or inflammation. Please carefully revise the results with CTC count cut-off and report the data in the tables (in this regard there is some recent evidence not reported, e.g. doi: 10.3390/biomedicines11010153).
Reply: Thank you for highlighting this point.
Changes in the text: None.

Question 6: The costs of the kit for detecting CTCs were not mentioned; the huge costs of these

analysis for the national health services are an important impediment to the routine spread in the clinical practice. How do the authors plan to overcome this obstacle?
Reply: We appreciate the reviewer's concern about the high costs of CTC analysis.
Changes in the text: None.

Question 7: Lung metastases represent a completely different disease and should be excluded from the analysis.
Reply: Thank you for this careful observation.
Changes in the text: None.

Reviewer B

Comments:
Authors present a study that characterizes circulating tumour cells (CTC) in different histologic classifications of nonsmall cell lung carcinoma based on demographics, radiologic features, genomic and protein expression. Using statistical tools including correlation analysis and logistic regression they identify features that are associated with CTC in each categories of NSCLC.
Comments
1.Authors improperly categorize immunohistochemistical features as genomic features. IHC suggests protein expression as distinct from genomic features which refers to gene/genomic mutations. These two techniques are regarded as distinct classes. authors should separate them.
Reply: Thank you for pointing this out. We agree that IHC reflects protein expression, distinct from genomic features, which pertain to gene or genomic mutations.
Changes in the text: None.

2. Authors suggest that it may be beneficial to add plasma CTC to the standard diagnostic workup for lung cancer but their study does not show how this would impact clinical decision-making. I believe this conclusion is not supported by the results of this particular study in a meaningful way.
Reply: We appreciate this observation. We agree that while our study highlights correlations between CTC levels and lung cancer characteristics, it does not directly measure the impact of CTC testing on clinical decision-making.
Changes in the text: None

3. The conclusion that the study elucidates the generation mechanism and regulation molecules of CTCs is not warranted from their findings. The findings are generally correlation, which do not imply a mechanistic cause.
Reply: Thank you for this important feedback.
Changes in the text: None

4. Authors need to present the summary statistics of the CTC detection results in each category i.e. present or absent, as well as range and median of CTC levels. Non of the tables show this.

Reply: Thank you for this suggestion.

Changes in the text: None