Peer Review File

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Reviewer A

The work analyzes the issue from an ingenious statistical approach, methodologically coherent. However, I believe that several aspects that I consider minor should be corrected:

P.5-l.8-9: "The methods of assessment and diagnostic criteria for diseases are available in the database."
I assume you are referring to Genetics of Iron Status (GIS) consortium: The authors should include a table specifying the diagnostic criteria, at least; I think the assessment methods can be dispensable.

Reply 1：Revise. Remove the description of this section.

Change in the text: We have modified our text as advised (see Page 5, line 9)

P.5-l.18-19: "The study was approved by institutional research ethics review boards and informed consent was obtained from the participants."
I assume that the authors are referring to the International Lung Cancer Consortium (ILCCO), in which case it is unnecessary to make this observation.

Reply 2：Revise. Remove the description of this section.

Change in the text: We have modified our text as advised (see Page 5, line 18-22)

P.5-l.20: Again, I think the authors should include a table defining the diagnostic criteria of the database they are going to use.

Reply 3：Revise. The table defining the diagnostic criteria of the database is located in the supplementary materials.
Change in the text: We have modified our text as advised (see Page 18, line 4)

P.7-l.30: The authors indicate that this is Table 2, when in fact these results are shown in Table 3.

Reply 4：Revise. The table order has been reversed.

Change in the text: We have modified our text as advised (see Page 17, line 9)

In P.8-l.32 the opposite occurs, logically.
(In the manuscript, the tables are presented as follows:
-Table 2 MR-Egger pleiotropy test for the instrumental variables associated with the four iron status biomarkers and the risk of lung cancer overall and the histological subtypes.
-Table 3 Causal effects between the four iron status biomarkers and potential confounding factors).

Reply 5：We are sorry for the wrong description. Revise.

Change in the text: We have modified our text as advised (see Page 8, line 32)

P.8-l.11-13: The authors must specify which marker each result corresponds to.

Reply 6：Revise.

Change in the text: We have modified our text as advised (see Page 8, line 12-14)

P.8-l.13-15: The authors make an erroneous, inverted interpretation of the result.

Reply 7：We are sorry for the wrong description. Revise.

Change in the text: We have modified our text as advised (see Page 8, line 15)

P.8-l.19-23: “Conversely, under the conservative approach, the only different result was that a correlation was found between the transferrin level and the risk of lung squamous cell carcinoma (OR: 1.05; 95% CI: 0.88–1.26; P=0.572).”
Contrary to what the authors state, this correlation is not statistically significant.
Reply 8: Revise. We are sorry for the wrong description. Revise.
Change in the text: We have modified our text as advised (see Page 8, line 19-22)

P.8-l.30-32: The authors must specify which marker each result corresponds to, in short form.
Reply 9: Revise.
Change in the text: We have modified our text as advised (see Page 8, line 32-34)

Reviewer B

In this study, the authors sought to assess the potential causality of serum iron status and lung cancer using the Mendelian-randomization (MR) method, selecting the

genetic variables for iron status from the Genetics of Iron showed higher serum iron status inversely related to the risk of lung squamous cell carcinoma but overall epidemiological evidence supporting the causality of iron status on lung cancer remains inconclusive and serum iron status had a negative causal effect on the risk of lung squamous cell carcinoma. Conversely, serum iron status was not found to be correlated with the risk of lung cancer overall and lung adenocarcinoma in general.

To me, the authors honestly conclude the study results as not conclusive.

I have a single comment, as follows:
Since cigarette smokers has high iron content in form of respiratory bronchiolitis/DIP or smoking related interstitial lung fibrosis (SRIF, into the smoker macrophages), I would expect significantly higher content of serum iron in this particular population. Is it correct? Please add a comment on that or add some comment in general, if there is body of evidence, concerning smokers and iron content.
Reply 1: Thanks for your comment. We have found some information that may be helpful to you. Ghio AJ et al published a study about cigarette smoke and iron homeostasis in 2008 (PMID: 18723436). In this research, after exposure of rats to cigarette smoke, increased lavage concentrations of iron and ferritin, serum ferritin levels, and nonheme iron concentrations in the lung and liver tissue all increased. This study suggested that cigarette smoke particles alter iron homeostasis, both in the lung and systemically. Thanks for your attention and we hope you have a great day!

Reviewer C
I thank the authors for sharing work with us.
Lung cancer needs too much work on it so each effort is valuable

As mentioned the causing factors about lung cancer is too many so selecting one and defining the risk might not descriptive, The smoking status, air pollution other cancer-causing factors needs purification during iron status.
(the authors mention it as a limitation but this needs more explanation)
The starting of the lung cancer and appearing as a mass lesion takes time (mostly for squamous lung cancer) also may differ according the stage of the disease , in the advanced stage we should be aware that the dietary in take would have lowered in the recent time (early stage of the disease) and got worse.So working (calculating) on similar stages with similar patient groups would be more additive

I might have missed the date of data collection if it includes the coronavirus pandemic time The ferritin levels were so high for many patients after the infection

How would you comment on The treatment plan and the treatment results (disease-

free survival, overall survival) and the iron level status
Do you have a group that had iron support and followed up in the treatment? Would you like to project a hypothesis or estimation? (would like to empower the discussion?

Thanks once again and good luck

Reply 1:

We are very grateful for the valuable comments. As for the bias about smoking status, air pollution other cancer-causing factors, this type of bias seems to be difficult to avoid in an observational study. A longitudinally and strictly designed large observational study may be more appropriate to study the causality between cancer-causing factors and cancer, to avoid associated bias. As for iron status and treatment results, unfortunately, we have not paid attention to the relevance between iron status and therapeutic effect in the coronavirus pandemic time. Firstly, serum iron status is not a must-check item for cancer patients. Next, ethical concerns also limit the study of serum iron status in cancer. Maybe, a retrospective study is feasible to explore the differences between serum iron status and therapeutic effect in cancer patients before or after the coronavirus pandemic time. Objective remission rate may be an appropriate indicator compared to DFS or OS, owing to its convenience and rapidity. Thanks for your attention and we hope it will be helpful to you!