Peer Review File

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Reviewer A

Manuscript summary:
In this study the authors assessed the clinicodemographic, CT, and PET-CT features of SMARCA4- deficient non-small cell lung carcinoma. The authors concluded that locally aggressive appearing lung lesions in male smokers should raise possibility of SD-NSCLC.

General comments:
The manuscript reviews a recently described subtype of lung carcinoma and the overall quality of the manuscript is high. The main limitation of the study is the presence of other recent manuscripts discussing this topic.

- **Reply:** Thank you very much for thorough comments on our study. As you suggested we have added recent works regarding SD-NSCLC and compared those results with our study in the discussion section.
- **Changes in the text:**
  Discussion
  According to our study, the majority of SD-NSCLC was manifested as relatively well-defined nodules or masses, showing lobulated contour and variable degree of enhancement. *This result shows difference from previous studies by Lou et al. (26), who reported 23 cases of SD-NSCLC in 2022, which mostly manifested as infiltrative masses and heterogenous densities with unclear margins (26). These image findings were in contrary to our study, and we believe such differences might be due to the fact that large proportion of our cohort were detected earlier phase during routine health check-up.*

Specific comments:
Title:
None.
Abstract:
1. Page 2 line 41-42: "In male smokers with peripherally located lung tumors showing invasion of adjacent pleura or chest wall regardless of small tumor size, radiologists should be concerned about the possibility of SD-NSCLC."- To make this statement true a comparison of the clinical and imaging appearance of SMARCA4- non deficient non-small cell lung carcinoma should be performed and show that the probability of these imaging features is significantly different between the 2 groups. I am not convinced this statement can be made without such a comparison since many SMARCA4- non deficient non-small cell lung carcinoma can also possess these features. The conclusion of this manuscript should focus on the difference between SD-UT and SD-NSCLC.

- **Reply:** Thank you very much for your comments on the conclusion of our study. As advised, we have added comments in the discussion regarding comparison between NSCLC and SD-NSCLC in the aspect of pleural invasion based on previous studies. Furthermore, since our study only included small number of participants, we have refrained from overly definitive expressions and have made the following modifications.
- **Change in the text:**

  Abstract

  Conclusion*: In patient with SD-NSCLC, there was tendency for male smokers, peripheral location and invasion of adjacent pleural or chest wall invasion regardless of small tumor size, when compared to SD-UT.*

  Discussion

  It has been known that SD-NSCLC shows highly aggressive behavior with vascular invasion and pleural metastasis (30), and high prevalence of pleural and chest wall metastasis might be due to SMARCA4 relation with trans-membrane glycoprotein CD44, which is closely related to metastasis (9, 31). *Furthermore, the incidence of pleural invasion was higher in our study when compared with the previous study of Heidinger et al. in terms of pleural invasion of pulmonary adenocarcinomas, according to CT features of primary tumor (32). In our study, the incidence of pleural invasion was observed to be 77.8%, whereas Heidinger et al. reported 24.6% in solid tumors and 4.7% in subsolid tumors (32). However, since our study included only small number of patients, further research with larger cohort would be necessary for more accurate comparison.*

Materials and Methods: None.

Results:

2. Page 8 line 190: “were ranged”- grammatically incorrect.

- **Reply:** Thank you very much for your comment on grammatic errors. We have made the change from 'were range' to 'ranged' as advised.
- **Change in the text:** After IV injection of contrast medium, measured net enhancement *values ranged from* 8 to 43 HU (median, 24 HU).

3. Page 8 line 206-209: “Presence of differentiated histology in SD-NSCLC is known to be a distinct feature from SD-UT, as SD-UT are generally associated with undifferentiated or rhabdoid morphology. Moreover, all nine cases showedTTF1 negativity, which is known to be found in 80% of SWI/SNF-deficient lung adenocarcinoma, which led to further evaluation of SMARCA4 (BRG1) and SMARCA2 (BRM).”-References?

- **Reply:** Thank you very much for your comment. We have added reference to the sentence regarding pathologic features, as follows *[Sauter JL, Graham RP, Larsen BT, Jenkins SM, Roden AC, Boland JM. SMARCA4-deficient thoracic sarcoma: a*

distinctive clinicopathological entity with undifferentiated rhabdoid morphology and aggressive behavior. Mod Pathol. 2017 Oct;30(10):1422-1432. doi: 10.1038/modpathol.2017.61. Epub 2017 Jun 23. PMID: 28643792.]

- **Change in the text:** Presence of differentiated histology in SD-NSCLC is known to be a distinct feature from SD-UT, as SD-UT are generally associated with undifferentiated or rhabdoid morphology **(25).**

Discussion:

4. Page 9 line 229-230: "CT and PET-CT imaging and clinicopathologic features of SD-NSCLC have not been clearly described up to date due to disease rarity." There are a few recent works published regarding this subject, for example- "Clinical, Radiological and pathological Features of SMARCA4 / BRG1-Decient Non-Small Cell Lung Carcinomas" by CY Lou and colleagues and "SMARCA4/BRG1–Deficient Non–Small Cell Lung Carcinomas: Imaging features at baseline according to TTF-1 status" by H. Žitnik and colleagues.

- **Reply:** Thank you very much for your kind comments. We have added references of recent works of SD-NSCLC as recommended.
- **Change in the text:** Due to the rarity of SD-NSCLC, only a very limited number of research regarding CT and PET-CT imaging and clinicopathologic features of SD-NSCLC have been reported to date (26, 27).

5. Page 10 line 251-252: "According to our study, the majority of SD-NSCLC was manifested as relatively well-defined nodules or masses, showing lobulated contour and variable degree of enhancement."- This differs from other case reports and series, and worth discussing. I presume that this can be related to the fact that a large portion of the cohort were diagnosed incidentally at an earlier phase.

- **Reply:** Thank you for the comments. We have added suggested contents with explanation, as follows.
- **Change in the text:** According to our study, the majority of SD-NSCLC was manifested as relatively well-defined nodules or masses, showing lobulated contour and variable degree of enhancement. This result was different from previous studies by Lou et al. (26). Lou et al. have reported 23 cases of SD-NSCLC in 2022, which mostly manifested as infiltrative masses and heterogenous densities with unclear margins (26). These image findings were in contrary to our study, and we believe such differences might be due to the fact that large proportion of our cohort were detected earlier phase during routine health check-up.

References:

As noted some literature does exist regarding this topic and should be added for completeness.

- **Reply:** Thank you very much for your comments on the references.
- **Change in the text:** As your advice, references were reviewed again, and several references were added.

Tables:
No specific comments.

Figures:
A comparison to appearance of SD-UT, would be of value for the readership if available.

- **Reply:** Thank you for your suggestion. Unfortunately, we do not have adequate number of pathologic-proven SD-UT cases in our institution to perform a comparative analysis between SD-UT and SD-NSCLC. However, we do believe this would be an excellent follow-up research opportunity.

Reviewer B

The SMARCA4 gene, belonging to the SWI/SNF family, is observed as a cancer suppressor mutation, notably in the realm of lung cancer. The focus of this study is on SMARCA4-deficient tumors, specifically analyzing the characteristics of SMARCA4-deficient non-small lung cancer (SD-NSCLC) in comparison with previously reported SMARCA4-deficient undifferentiated tumors (SD-UT). The analysis encompasses clinical background, imaging examinations through CT and PET-CT, as well as diagnostics and treatment approaches. This cohort study involves nine patients with pathologically diagnosed SD-NSCLC. The findings suggest that these patients, predominantly male with a history of smoking, often exhibit distinct radiological features, including well-defined boundaries and frequent chest wall invasion. While the study presents interesting insights into common characteristics within the SD-NSCLC cohort at this facility, it's worth noting that there is still room for discussion regarding the accuracy of these conclusions.

Major Points:

1. This analysis focuses on nine patients diagnosed pathologically with SD-NSCLC. A key question is whether the findings from these nine patients can be generalized. To address this, further analyses are needed. Firstly, how many cases does this cohort represent in total? The frequency of SD-NSCLC is mentioned to be about 5-10% of all NSCLC cases (Line 83), but it's unclear whether the frequency in these nine patients is similar or different from this range. If different, an explanation is necessary.

- **Reply:** Thank you for your insightful suggestion. In fact, we conducted a comprehensive search within our institution's pathologic reports for the term "SD-NSCLC" and identified a total of 9 patients. Despite anticipating a higher prevalence aligning with reported frequencies of 5-10% in NSCLC, we encountered only limited number of cases. This incongruity may stem from potential miscommunications in terminology or an evolving landscape in the field of pathologic diagnosis.

2. Related to the above point, it would be beneficial to include SD-UT patients in the same cohort for comparative analysis. Comparing SD-UT and SD-NSCLC within the

same cohort could reduce diagnostic bias, as opposed to only comparing SD-UT with previously reported data.

- **Reply:** We appreciate your critical feedback. We share the conviction that comparing SD-NSCLC with SD-UT patients within the same cohort from our institution would enhance the study's robustness and minimize potential bias. Unfortunately, our current dataset comprises no more than 3 cases of SD-UT. To address this limitation, we are actively working on accruing additional cases to facilitate a comprehensive comparative analysis. We are grateful for the suggestion and acknowledge the potential for an excellent research opportunity as a follow-up paper. Thank you for your valuable input.

3. SMARCA4 mutations are known to have inclusive relationships with other co-mutations like KRAS, STK11, and KEAP1. The CT image characteristics of KRAS lung cancer, for example, seem to overlap with those described in this study (Eur Radiol. 2016 Jan;26(1):32-42). It's necessary to analyze at least KRAS mutations in these nine cases to discern whether the observed effects are solely due to SMARCA4 mutations or influenced by other co-mutations.

- **Reply:** Thank you for your thorough suggestion. Five patients in our study underwent immunohistochemical staining for p53/p63 and all patients showed negative results. Unfortunately, KRAS mutations were not assessed in any of the nine patients with SD-NSCLC included in our analysis. We acknowledge the significance of investigating the potential impact of KRAS mutations on imaging features, as highlighted in your comments. This presents a valuable avenue for subsequent research, and we appreciate your keen insight. Once again, thank you.

4. Line 108 states, “we found nine patients with pathological confirmation of SD-NSCLC.” I'm interested in the selection criteria used at your facility for SMARCA4 or SMARCA2 IHC evaluation. For instance, is it routinely performed in all cases of TTF1-negative NSCLC? If there are cases with the identified characteristics of male gender, smoking history, and radiological features, which have not yet undergone IHC evaluation for SMARCA4, including these in a further analysis could make your conclusions even more compelling, especially if a high number of SMARCA4-negative cases are identified.

- **Reply:** Thank you for the suggestion. We do not perform routine SMARCA4 or SMARCA2 IHC evaluation in all cases of TTF1-negative NSCLC. After request to our pathologist, we found that only limited number of cases with eccentric pathologic features (i.e., excessively pleomorphic) underwent IHC evaluation for possible SMARCA4/2 deficiency.

Minor Points:

1. One aspect of the study background highlights the difficulty in differentiating between SD-NSCLC and SD-UT, emphasizing the importance of this distinction for

choosing appropriate treatment regimens. However, both conditions appear to have poor prognoses, with early stages typically being candidates for surgery and advanced stages for chemotherapy. Additionally, there is no consensus on the effectiveness of immune checkpoint inhibitors (ICIs) in these cases, suggesting minimal differences in treatment approaches. The manuscript lacks sufficient details regarding the specific differences in treatment regimens between these two conditions. Therefore, I recommend adding more information on this in the Discussion section to clarify the significance and importance of your study.

- **Reply:** Thank you very much for your valuable advice. We have added recent studies regarding ICI in both SD-NSCLC and SD-UT. Thanks to the advice you provided, the overall completeness of the manuscript has been significantly improved.
- **Change in the text:**

  Discussion

  Regarding the relationship between SD-NSCLC and SD-UT, recent study by Lin et al. demonstrated survival benefit of immune checkpoint inhibitor (ICI) comparing to traditional chemotherapy in SD-UT, and the prognostic disparity between SD-NSCLC and SD-UT under different treatment settings (35). ICI-based treatment significantly improved progression-free survival than chemotherapy in the first-line treatment of SD-UT (35). Moreover, patients of SD-UT and SD-NSCLC receiving ICI-based regimen as first-line treatment had significantly longer median OS than those having ICI-based regimen in latter line or no ICI treatment throughout clinical courses (35). These results indicated the promising efficacy of ICI in metastatic SD-UT, and its optimal effect may be achieved when used early in clinical courses (35). Regarding the prognostic disparity, no significant survival differences were observed in progression-free survival between SD-UT and SD-NSCLC of under same treatment settings (35). However, some studies showed limited efficacy of ICI in SD-UT. Gantzer et al. reported that SD-UT mostly had an immune-desert tumor microenvironment (36). Only 1 out of 4 patients in Gantzer's cohort turned out to have immune-rich tumor microenvironment responded to ICI (36). However, despite multimodal treatment therapy including chemotherapy and surgery, the clinical outcomes of most patients with SD-NSCLC and SD-UT have been discouraging to date (18, 35, 37).

Reviewer C

The authors reviewed characteristics of SMARCA4 deficient NSCLC. They retrospectively evaluated the clinicodemographic and imaging features. They found that SD-NSCLC is commonly seen among male smokers with peripheral location, and frequent pleural invasion.

The manuscript is well written, and results are easy to understand.

However, the number of patients is too small to draw any conclusion. Additionally, the characteristics of SD-NSCLC they found are common in NSCLC.

If the authors add rigorous comparison with other disease entities, the manuscript can

draw interest of readers.

- **Reply:** Thank you very much for your comments on the conclusion of our study. As your advice we have added comments in the discussion, regarding comparison between common NSCLC and SD-NSCLC in the aspect of pleural invasion based on previous studies.
- **Change in the text:**

  Discussion

  It has been known that SD-NSCLC has highly aggressive behavior with vascular invasion and pleural metastasis (30), and high prevalence of pleural and chest wall metastasis might be due to SMARCA4 relation with trans-membrane glycoprotein CD44, which is closely related to metastasis (9, 31). *Furthermore, incidence of pleural invasion was higher in our study, compared with the previous study of Heidinger et al. regarding pleural invasion of pulmonary adenocarcinomas, according to CT features of primary tumor (32). In our study, the incidence of pleural invasion was observed to be 77.8%, whereas in the previous study by Heidinger et al., it was reported as 24.6% in solid tumors and 4.7% in subsolid tumors (32). However, since our study included only small number of patients, additional research with larger cohort would be necessary for an accurate comparison.*