Peer Review File

Article information: https://dx.doi.org/10.21037/jtd-23-1811

Reviewer A

This submission makes no sense at all for the following reasons:

Non-small cell lung cancers (NSCLC) may express neuroendocrine cell markers but you have to look for them prospectively. Their expression varies between different markers: for instance, 8.6% for N-CAM and 0.4% for chromogranin (Am J Surg Pathol, 2007).

In this submitted study is retrospective and based on publicly available data (SEER). In the majority of the cases, the pathologists have most probably NOT looked prospectively for neuroendocrine differentiation with a large battery of markers.

The authors should have reexamined every case for this type of differentiation, which of course would be unrealistic.

Reply1: We greatly appreciate your professional review of our article. Thanks to your valuable suggestions, we have the following response. According to the WHO classification criteria, a tumor with neuroendocrine differentiation is diagnosed when a neuroendocrine marker is clearly positive in the tumor tissue and the number of positive cells does not exceed 50%. Therefore, it is not necessary for all neuroendocrine measures to be positive. In practical clinical practice, pathologists also confirm the diagnosis of adenocarcinoma with neuroendocrine features based on diagnostic criteria. At present, the prediction of postoperative survival for lung cancer is based on the biological characteristics. More and more researchers are devoted to studying neuroendocrine differentiation in lung cancer and analyzing its relationship with the malignant degree. This is done in order to guide the treatment and prognosis of lung cancer. Therefore, we hope to attract enough attention to this kind of patients through a retrospective analysis of the SEER database. Thank you very much for your comments, and we hope that our answers will satisfy you. I wish you a happy life and work.

Changes in the text: None

Reviewer B

This manuscript is a retrospective analysis of the survival and prognosis of adenocarcinoma vs adenocarcinoma with neuroendocrine differentiation, and neuroendocrine cancer. Please see my comments below:

1. Previous publications have termed ADE_ned as LANED (Lung Adenocarcinoma with Neuroendocrine Differentiation). Is there a reason for using a different convention in this manuscript?

Reply1: We sincerely thank you for your careful review of our manuscript. In response to your question, in writing, we mainly want to better distinguish adenocarcinoma (ADE), so we use adenocarcinoma with neuroendocrine differentiation, abbreviated as ADE_ned.

Changes in the text: None

2. Is there any data on the proportion of patients who received immunotherapy.
Reply2: Thank you very much for your thoughtful question. It is regrettable that specific data on patients receiving immunotherapy is not available, making it impossible to analyze its effect on immune efficacy.
Changes in the text: None

3. Following propensity matching, the overall survival (OS) in graph C suggests that the mortality between adenocarcinoma and NEC is the same at about 60 months (see the intersection) and worse for adenocarcinoma thereafter. Is there a reason for this?
Reply3: We appreciate the questions raised. In response to your questions, we propose the following explanations. Figure C compares the overall survival between NSE and ADE. However, it is important to note that OS indicators also include deaths caused by non-cancer causes, which may result in a worse prognosis for ADE after 60 months.
Changes in the text: None

4. Is there any data available on the subtypes of neuroendocrine tumors for the NEC group?
Reply4: Sincere thanks should be given to the reviewer for the constructive comments and suggestions. The responses to the comments are given below. Because all neuroendocrine tumors were combined into the NEC group for statistical analysis, we did not further compare the prognosis of specific subtypes.
Changes in the text: None

5. Consider Kaplan-Meier curves based on stage for the three different tumors.
Reply5: Thank you for your valuable advice. At the time of statistical analysis, because there were only 316 cases in the ADE_ned group. Therefore, if the survival was further compared according to stage, there were too few cases in each subgroup.
Changes in the text: None

Reviewer C

The paper investigated the prognosis of patients with ADE, NEC or ADE_ned. NE differentiation can be detected by immunohistochemistry in non-small cell carcinomas lacking any neuroendocrine morphology. Although whether NE differentiation impacts clinical outcome is controversial, it is commonly believed that NE differentiation does not bear a clinical impact.
This paper however found that patients diagnosed with ADE_ned had significantly lower survival rates compared to patients with lung ADE or NEC. The findings are interesting.

1. Line 76-77, “with a minimum threshold of more than 50% positive cells; otherwise, it is defined as tumor with neuroendocrine differentiation”
Please add references to justify the “50%” criteria or rephrase the sentences.

Reply1: Special thanks to you for your good suggestions. We have cited the relevant references before (see Page3, line 78).
Changes in the text:None

2 Separation LCNEC from adenocarcinoma ADE-ned can be challenging and require a set of criteria. Widely varied diagnostic criteria could impact diagnosis consistency across studies utilizing separate institutional data, and subsequently affect the prognosis results. I noticed that the data in this paper were extracted from the SEER database. I wonder if you could briefly discuss the issue and the potential limitation of using SEER classification as the primary data source.
Reply2: We would like to express our heartfelt thanks to the reviewers for their constructive comments and suggestions, and will reply to the reviewers' comments as follows. The main basis for distinguishing neuroendocrine tumors from tumor cells with ND is whether some tumor cells with neuroendocrine differentiation in the tumor tissue are only a concomitant component of the tumor tissue. According to the WHO classification criteria, a neuroendocrine tumor is diagnosed when a neuroendocrine marker is positive in the tumor tissue and the number of positive cells exceeds 50%. Otherwise, the tumor is defined as neuroendocrine differentiation. Cancer with neuroendocrine differentiation is a concurrent component of cancer, distinguished from neuroendocrine tumors by the presence of differentiated neuroendocrine cells that make up less than 50% of the tumor components and are scattered in the form of single cells or cell nests. With respect to the potential limitations of using SEER classification, it is mainly not known which specific diagnostic metric is used in this pathological diagnosis.
Changes in the text:None

3. Minor issues
Line 227 “synucein” should be “synaptophysin”?
Line 228 Change “neural cell adhesion molecule (NCAM)” to “neural cell adhesion molecule (NCAM, CD56)”.
Reply3: We feel sorry for our carelessness. In our resubmitted manuscript, the typo is revised. Thanks for your correction (see Page8, line 227, line 228).
Changes in the text:we have modified our text as advised (see Page8, line 227, line 228).