Peer Review File

Article information: https://dx.doi.org/10.21037/tlcr-23-855

<<Reviewer A>>
Comment (general):
Authors retrospectively reviewed 1,733 patients with suspected lung cancer
using tumor markers as index tests and clinical diagnosis by the physician as
the reference index. Results are that the combination of three tumor markers, namely CEA, CYFRA, and NSE provided the better diagnostic test accuracy than panel of 6 tumor markers. Limitations have been stated. The paper is well written and logically organized. Some points can improve the quality of the paper and has to be recommended:

Reply (general):
Thank you very much for your numerous helpful comments. Please find below our point-by-point responses.

Comment 1:
can you discuss the role of cytology and histology in which tissue markers can have different protocols and performance. The issue is fundamental, specifically in a context in which materiale can be scarce (please quote PMID: 34478240, PMID: 33644101)

Reply 1:
Following your suggestion, a paragraph was added with the two recommended references. Thank you.

Changes in the text:
The following paragraph was added as the second last paragraph in the discussion section.
In the diagnosis of lung cancer, histopathological and cytopathological examinations through methods such as bronchoscopy, video-associated thoracic surgery, CT-guided lung biopsy, and thoracentesis have been the standard. However, in the real world, it is not always possible to obtain pathological specimens, necessitating the exploration of lung cancer possibilities through alternative methods. Serum tumor markers have been one of the traditional alternatives, but recent years have seen a growing interest in new diagnostic approaches that accommodate a variety of specimens, including liquid biopsies, facilitated by next-generation sequencing and new genetic biomarkers.

Comment 2:
please provide a graphical abstract which can show the flow of your message in a picture.

Reply 2:
A graphical abstract was made to show the flow of our message in a single picture.

Changes in the text:
A graphical abstract is attached.

<<Reviewer B>>
Comment (general):
The manuscript entitled “Improved diagnostic accuracy with three lung tumor markers compared to six-marker panel” is a good work, written in a clear and fluent manner. The theme and objectives are explicit from the beginning. However, the purpose proposed by the authors to determine the minimal necessary combination of tumor markers cannot be correctly achieved without having available histopathological type and stage of patients with lung cancer. The lack of these data does not allow correct identification of the

patient with lung cancer, which is not always clinically diagnosable without the aid of other diagnostic parameters. Furthermore, considering the link of some markers to a precise histology of lung cancer and its diffusion stage, the lack of these data does not allow a correct comparison with other works such as that of Molina et al to state that a panel of only three markers can be more useful than one of 6 tumor markers. Therefore I suggest a review of the data to associate the histology and stage of the lung tumors with the tumor markers, so it is possible to have a more relevant statistical result for comparison.

Reply (general):

As noted by the reviewer, data on histological types and disease stages are crucial factors when discussing the sensitivity and specificity of tumor markers. This is because tumor markers that are matched to specific histological types of lung cancer can increase sensitivity, and advanced disease stages can also raise sensitivity. When individual patient histology and disease stage data are available, it's possible to determine the absolute sensitivity and specificity of tumor markers.

However, the sensitivity and specificity of tumor markers are significantly influenced by lung cancer histology, disease stage, tumor size, lymph node involvement, presence of metastasis, and therapeutic interventions. Therefore, discussing the “absolute” sensitivity and specificity of tumor markers for lung cancer “in general" is not very meaningful. Our analysis is neither for the absolute diagnostic test accuracy nor to compare the panel of three markers with Molina’s panel. It focuses on the comparison between panels of six and three tumor markers. This comparison can be analyzed even without data on lung cancer histology or disease stage. The diagnostic capabilities of both the six and three marker panels decrease at lower disease stages, but the relative relationship is preserved.

Unfortunately, the dataset available to us, as stated in our previous manuscript, lacks data on lung cancer histology, disease stage, tumor size, lymph node involvement, and presence of metastasis, preventing further analysis.

The key message we want to convey is that measuring six tumor markers may increase sensitivity but at the expense of lower specificity. Some clinicians, fearing to miss lung cancer, may opt to check as many tumor markers as possible, sacrificing specificity for increased sensitivity. Normal values for tumor markers are often set to include 95% of healthy individuals. A single tumor marker can produce a 5% false positive rate, and testing six tumor markers can nearly increase the false positive rate to 30%. False positives for the entire tumor marker panel can lead to unnecessary invasive procedures with significant risks, such as bronchoscopy, VATS biopsy, or CT-guided lung biopsy, making it crucial to keep an appropriate number of tumor marker measurements.

While we stated that a panel combining CEA, CYFRA, and NSE offers higher diagnostic capabilities than a six-tumor marker panel, our intention is not to recommend this specific combination. Instead, our message is a caution against the excessive number of tumor marker items leading to an increase in false positives. To ensure our stance is clearly communicated, we have revised the abstract, highlight and conclusion.

We are grateful for the thorough review and valuable comments.

Changes in the text:

The followings were amended. The conclusion section of the abstract. The last sentence of the Highlight. The conclusion section of the main text.

<<Reviewer C>>

Comment (general):

The authors have examined a panel of tumour markers in a retrospective lung cancer study at one hospital. They argue that there is a benefit from using three markers instead of six.

The published evidence for the use of any one of these tumour markers in lung cancer is poor, and also their own AUC figures do not support the use of these markers in lung cancer studies.

The design of this biomarker study has to be questioned; a cohort study involving only one location with no ‘control population’ tested.

This is not the recommended approach for testing lung cancer biomarkers.

Reply (general):

We agree with several points raised by the reviewer. First, we concur with the observation that the AUC in our analysis is low, indicating that the diagnostic accuracy of tumor markers for lung cancer is insufficient. It is widely known that tumor markers inherently lack sufficient performance for diagnosis and are not suitable for screening purposes like health checkups. Typically, tumor markers should be used for confirming recurrence or monitoring the progression of cancer. What we are advocating in this paper is not the “absolute” diagnostic performance of a three-marker panel. Our discussion focuses on comparing the relative sensitivity and specificity between panels of six and three tumor markers. We believe there is significance in this comparison, even if the absolute AUC values are low.

Furthermore, we agree with the reviewer's suggestion that a control group is necessary in studies of sensitivity and specificity. As stated in our manuscript, our analysis included 779 lung cancer patients and 954 control non-lung cancer individuals. We are pleased to find agreement with the reviewer on the necessity of including controls in the analysis.

Additionally, the reviewer has pointed out that our study is a single-center study. We acknowledge the limitations of a single-center study, which we have documented in the Limitations section of our paper.

We are grateful for the thorough review and valuable comments.

Changes in the text:

The last sentence of the Discission section (limitation section).