

## Peer Review File

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### Reviewer A

Congratulations to the authors for their effort in filling the knowledge gaps in the current literature on investigating malignant pleural effusions.

**Reply:** Thank you for this comment.

Please refer to my comments for each section below.\

Introduction:

Well presented setting the background for the study.

Suggest including the abbreviation expansion for BPE in the introduction.

**Reply:** This has been added.

**Changes in the text:** Page 5; Line 73.

Methods:

The methods comply with the STARD standards.

Recruitment of participants, diagnostic criteria for MPE and other BPE are well presented. It is presumed that TPE, PPE, and HFPE are considered under the main category of BPE. Suggest clarifying that.

**Reply:** We have modified the text as suggested.

**Changes in the text:** Page 6; Lines 124–126.

Given this is diagnostic accuracy study, suggest including the assay specifications for the index test including LOD, LOQ, CV at the limits adopted by the authors for diagnostic purposes (eg: 40 pmol/L).

**Reply:** We have added these analytical characteristics to the revised manuscript accordingly.

**Changes in the text:** Page 7; Lines 131–135.

Statistical analysis is appropriate for a diagnostic accuracy study.

**Reply:** Thank you for your comments.

**Changes in the text:** None.

Results:

Suggest providing a cross tabulation of the index test results against those of the reference standard for the selected cutoff limit (40 pmol/L) for sensitivity and specific calculation.

**Reply:** We have added the absolute numbers of TP and TN in the main text, instead of a cross-table.

**Changes in text:** Page 8; Line 171.

Discussion:

Results discussed with appropriate reference to existing evidence.

**Reply:** Thank you for your positive comments.

**Changes in the text:** None.

Authors claim that the index test has a high diagnostic accuracy for SCLC-MPE at a cutoff limit of 40 pmol/L.

Diagnostic accuracy is expressed as the proportion of correctly classified subjects among all subjects: Accuracy = (true positives + true negatives) / (total)

At a cutoff limit of 40 pmol/L, the specificity has suffered at the expense of improving sensitivity.

Therefore, invariably the number of true negatives will suffer due to low specificity thus reducing the diagnostic accuracy of the index test at this limit. Thus, this claim is inaccurate based on the results. Please amend this as appropriate.

If the intent is to have a better diagnostic accuracy with a rule in, suggest selecting a cutoff limit with a better specificity parameter.

**Reply:** We did not report the accuracy of an index test with a cross table [(TP + TN)/N] because the numbers of TP and TN are threshold-dependent. Therefore, accuracy only reflects the accuracy of an index test at a given threshold. Alternatively, the AUC is a widely used metric to estimate the diagnostic accuracy of an index test because it is threshold-independent [Clin Chem. 2012;58(9):1292-1301.]. We concluded that proGRP has high diagnostic accuracy for SCLC because it has an AUC of 0.90.

As shown in Figure 2, some patients with other types of MPE and BPE also had high proGRP levels (>500 pg/mL); therefore, there was no threshold with a specificity of 1.00.

**Changes in the text:** Page 8; Lines 181–184.

Likelihood ratios above 10 and below 0.1 are considered to provide strong evidence to rule in or rule out diagnoses respectively in most circumstances. Considering +veLR was only 2.6 at this level with a -veLR of 0.40 pmol/L would be more appropriate for rule out SCLC rather than for rule in, which is correctly identified by the authors and discussed thusly.

**Reply:** Thank you for your positive comments.

**Changes in the text:** None.

Authors have appropriately identified limitations of the study and limitations of the index test.

**Reply:** Thank you for your positive comments.

**Changes in the text:** None.

Adopting 2 cutoff limit for rule in and rule out of SCLC-MPE would allow improving the sensitivity and specificity in each situation. Especially as diagnostic specificity is discussed with regards to the index test, it would be more appropriate to provide a cutoff limit with better specificity parameters in the results.

**Reply:** As shown in Figure 2, some patients with other types of MPE and BPE also had high proGRP levels (>500 pg/mL); therefore, there was no threshold with a specificity of 1.00. Therefore, we only reported the threshold with a sensitivity of 1.00.

**Changes in the text:** None.

Conclusion:

Statement on high diagnostic accuracy of the index test for SCLC-MPE at 40 pmol/L is statistically inaccurate. Suggest correcting as appropriate depending on how authors decide to change the result section.

**Reply:** We concluded that proGRP has a high diagnostic accuracy for SCLC because it has an AUC of 0.90.

**Changes in the text:** None.

### **Reviewer B**

You make a good argument about need for less invasive approaches to characterize malignant pleural effusions. This is a diagnostic challenge for many clinicians.

Small number of patients with SCLC in the Changshu cohort is a limiting factor of your study as noted.

Are you suggesting that proGRP may be used to exclude SCLC associated pleural effusion? If so, how would that be beneficial to a clinician and the patient. I would expand on that remark.

**Reply:** Thanks for the comment. Indeed, a low proGRP level seems to reasonably exclude SCLC as the cause of pleural effusion. However, it should be noted that any tumor marker evaluated in pleural fluid is only indicative of the possible malignant nature of an effusion and does not obviate the need to demonstrate this possibility cytohistologically. However, in patients with lung tumors and associated pleural effusions who are in a life-threatening condition, where it has not yet been possible to ascertain a precise etiology, the finding of a proGRP of < 40 pg/mL would favor the use of empirical systemic oncologic therapy directed at NSCLC rather than SCLC. This idea has been added to the Discussion section.

**Changes in the text:** Pages 9–10; Lines 213–221.