BASIC REPORTING

The study addresses an important clinical problem on detection of malignancy in serous effusions; however, the manuscript requires substantial improvement in terms of background and justification with literature, data interpretation, and language. At present, the introduction and discussion do not provide sufficient context. English language and coherence of the text need major revision to ensure that the findings are accessible to an international audience.

1. Your most important issue is incorrect use of the term *novel biomarker* which reflects overstating of your finding and is not justified by the presented evidence. Not only can HFCs not be considered a biomarker, as they are also expressed in non-tumor cells in effusions, but the definition of a novel biomarker requires extensive investigations, which are not present in your work. The outcome of this study is not a novel biomarker, and this claim should be avoided in whole manuscript.

2. The next most important item is lacking an appropriate citation to prior works. For example, Wu et al. (Int J Lab Hematol. 2019. 41(4):509-512), applied a similar strategy using combination of CEA and HFCs for diagnostic purposes. Please explain how your work is different.

3. Your introduction needs more detail and references:

   3.1. Please clarify “other conditions” and provide references in Line 47.

   3.2. You are highlighting the problems with exfoliative cytology and advantages of *Sysmex XN-10* as a solution. However, this section lacks detailed comparison based on the literature. Readers need more detail on specificity and sensitivity of *Sysmex XN-10* compared to exfoliative cytology. In this context, please specify most relevant parameters to malignant effusion detection.

   3.3. In Line 50, you state that exfoliative cytology is affected by the quality of collected material, but you do not explain how this differs from Sysmex XN-10. Both methods use effusion/liquid biopsy material. Please clarify this distinction.

   3.4. Please indicate how low sensitivity improved by Sysmex XN-10.

   3.5. Please provide more explanation and background on how following markers: WBC, RBC, CEA, LDH, and ADA are related to malignant effusion diagnosis.

3.6. Please provide rationale for focusing specifically on lung cancer as one of your major results.

3.7. Please provide more explanation and background on CEA.

3.8. Please provide systematic comparisons and cite prior studies regarding conventional cytology examination in Line 196.

3.9. In Lines 199–201, I suggest that, rather than comparing various translational research methods, compare Sysmex XN-10 with clinical diagnostic methods currently used in liquid biopsy. This will establish stronger clinical relevance.

3.10. "Faster detection speed "in Line 225, needs elaboration and supporting evidence.

3.11. In Line 250, the limitations section is incomplete. Using Sysmex XN-10*,* mesothelial cells and macrophages may be counted as high-fluorescent cells, affecting the accuracy of the cell count results. You only investigated the values of HFC% and HFC# detected by the instrument, without accounting for this inaccuracy. This impacts the diagnostic efficiency for distinguishing benign and malignant pleural effusions.

3.12. In line 256, you must precise that HFC *in combination with CEA* provide a reliable basis for rapid screening.

4. The manuscript requires significant editing for clarity and coherence. Some sentences, as well as titles of results, and figure legends are unclear and are difficult to understand. I recommend professional editing.

5. Figures:
   Thank you for providing the raw data files. However, figures need improvement based on the following remarks:
   - I recommend restructuring Figure 1 to interpret easier. Specifically, merge panels A and B Include following markets: HFC#, HFC%, CEA, LDH, HFC#+CEA, HFC%+CEA
   - Avoid the three-variable (HFC# + HFC% + CEA) model in both figure 1 and figure 2. HFC# and HFC% are strongly collinear. Presenting that model in the main figure is confusing.
   - Improve figure legends for both figure 1 and figure 2. This part needs clearer wording and explanation.

EXPERIMENTAL DESIGN

The research presented in this manuscript stands well within the aims and scope of the journal.

The research question is well defined, clinically relevant, and meaningful. The authors make effective use of a large dataset compromising of 978 clinical effusion samples, which strengthens the statistical power and clinical relevance of their findings.

The study contributes by evaluating the diagnostic value of HFCs in malignant serous effusions and demonstrates that, when combined with CEA, diagnostic accuracy improved.

The methods are described with sufficient detail, and overall, the manuscript provides valuable insight into the potential clinical utility of HFCs for diagnosing malignant effusions.

VALIDITY OF THE FINDINGS

The conclusions are not appropriately stated and are not fully supported by the results. Claims of *a novel biomarker* are overstated*;* establishing novel biomarkers requires an extensive experimental validation, which is not present in this manuscript. Moreover, the strategy presented here is not new, as both HFCs and the combination of CEA with HFCs have already been reported in the literature.

That said, this study makes meaningful contribution by validating HFCs as an indicator of malignancy in a larger patient dataset than previously published. All underlying data are robust, statistically correct, and well controlled.

In conclusion, there is a weakness in written and interpreting data (noted above) which should be improved before acceptance.