Peer Review File

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Reviewer A Comments

The manuscript focuses on a novel field where liquid biopsy may be approached. Accordingly, the manuscript requires major changes to be suitable for publication on this journal.

1. In the text, I would recommend discussing in details the new frontiers of liquid biopsy. In particular, the authors should discuss technical approaches, clinical findings of most promising liquid biopsy- related data. In addition, a section with the further application of liquid biopsy derived analyses should be added.

Reply 1: We thank the reviewer for the suggestion and have added a section to discuss the commonly adopted technical approaches of liquid biopsy analysis and the associated advantages and disadvantages (in the paragraph titled 'Analytical methods and pre-analytical considerations'). In addition, we have added a section to discuss the further application of liquid biopsy on multi-cancer early detection in the new paragraph titled 'Emerging applications'.

Changes in the text: A new paragraph titled 'Analytical methods and per-analytical considerations' has been added to discuss the commonly adopted technical approaches for liquid biopsy (pages 6-7, lines 108 – 132). Another new paragraph titled 'Emerging applications' has been added to discus the further application of liquid biopsy on multi-cancer early detection (pages 12, lines 251 – 258).

2. Please, could the authors review all the figures and tables? In my opinion, they should improve quality of these tools.

Reply 2: We would like to thank the reviewer for the suggestion. We have revised the two figures and improved the resolution.

Changes in the text: We have improved the figure resolution of the Figure 1 and Figure 2 as suggested.

Reviewer B Comments

The authors presented a systematic review of studies investigating the role of circulating tumour DNA analysis in the detection of lung cancer. The study is well-designed and presented and

provides valuable information on this important topic. I have a few suggestions that might improve the quality of this article.

Major suggestions

1. Consider adding a table summarising data presented in the Results, such as studies' size, target population (e.g., only early vs all lung cancers, pantumour populations), control groups, and methods employed, using internal or independent validation cohorts.

Reply 1: We thank the reviewer for the suggestion. Indeed, we have already included a table listing the information suggested. We have now highlighted the inclusion of the summary table and explicitly stated the information on the study size, target population, control group used, methodology in the table (i.e. Table 1).

Changes in the text: We have modified our text to highlight the summary table (see pages 8 – 9, lines 162 – 166)

2. It would be advisable to provide in the Discussion the lack of clinical implementations of circulating DNA technologies in clinical practice, explain the reasons for that, and consider mentioning the lack of clinical utility validation of DNA screening methods. In conclusions, present perspectives for further developments.

Reply 2: We thank the reviewer for the suggestion. We have now discussed the hurdles to the clinical implementation of circulating DNA analysis especially for early cancer detection. We have mentioned that the lack of validation (resource demanding) may be one limiting factor to clinical implementation.

Changes in the text: The discussion mentioned above has now been included in the revised first and second paragraphs of the Discussion section (pages 12 – 13, lines 260 – 280).

3. Discuss the study limitations shortly.

Reply 3: With the reviewer's suggestion, we have now discussed the study limitations in the Discussion.

Changes in the text: We have added the study limitation of using only one search engine as stated above in the revised Discussion section (see Page 15, lines 312 – 315).

Minor suggestions

1. A semantic issue. The authors use the term "early detection of lung cancer," which may imply early lung cancer detection. Actually, most of the presented studies were designed to detect lung cancer (or also other tumours) at the asymptomatic and not a strictly early phase. Indeed, as expected, testing efficacy was generally in more advanced stages. This also has clinical implications – what matters in lung cancer is its detection at a curable rather than in an advanced stage.

Reply 1: We agree with the reviewer's comment on the use of the term 'early detection of lung cancer. Therefore, in lines 174 – 176, we have used the term 'detection of lung cancer only' to replace 'early detection of lung cancer only'. As already mentioned by the reviewer, most of these studies were retrospective case-control analyses of lung cancer and control samples and the diagnostic performance metrics were better for advanced stage cancer as expected. Therefore, in order to facilitate the reader's interpretation on the performance of the various methodologies in the different studies, we have explicitly stated the stage distribution, if available, of cancer cases in the summary table (Table 1). In addition, we have also specifically mentioned such implication for evaluating the performance for detection of lung cancer at an early, curable stage (Discussion, page 13, lines 271 – 280).

Changes in the text: In lines 174 – 176, we have used the term 'detection of lung cancer only' to replace 'early detection of lung cancer only'. The paragraph in the Discussion (page 13, lines 271 – 280) is modified to address to the reviewer's comment on the term 'early detection of lung cancer)

2. The authors use the terms: "sets," "datasets, "cohorts," and "groups interchangeably." This should be unified or, if they indeed differ, explained. The same concerns "testing" and "validation" datasets.

Reply 2: We thank the reviewer for the suggestion. While we have initially kept these terms as originally used in the individual studies, we have now aligned the use in both the main text and the table of our current systematic review.

Changes in the text: The terms mentioned by the authors were now aligned as suggested by the reviewer.

Reviewer C Comments

The manuscript has several limitations. A few are listed as below.

1. The authors aim to investigate the value of ctDNA for Lung cancer screening, but majority of the studies included are not Lung cancer screening studies. In their methods they say, studies with known history of lung cancer were excluded, but they still included several studies with known lung cancer.

Reply 1: We thank the reviewer for the comment. As pointed out by the reviewer, the majority of studies that evaluated the performance of ctDNA analysis for early detection of cancer detection were of case-control study design. These studies include cases with a confirmed diagnosis of lung cancer. As mentioned in the 'Methods' section, we excluded studies that evaluated the

performance of ctDNA analysis for detection of recurrence or surveillance, therefore we stated that 'studies … with no known history of lung cancer were included'. With the reviewer's comment, we have now revised the statement as 'Studies which evaluated ctDNA analysis for lung cancer detection in symptomatic / asymptomatic patients with no known previous history of lung cancer (primary but not recurrent cancer) were included.'

Changes in the text: In the Methods section, the statement is now revised as 'Studies which evaluated ctDNA analysis for lung cancer detection in symptomatic / asymptomatic patients with no known previous history of lung cancer (primary but not recurrent cancer) were included.' (pages 7 – 8, lines 140 – 142).

2. They only used one search engine 'PubMed' which is not sufficient and could have missed several important studies. For a good SR, at least 3 search engines should be included e.g., MEDLINE, EMBASE, COCHRANE, etc.

Reply 1: We thank the reviewer for the comment. We understand that we may have missed a few studies by using only one search engine. Nevertheless, we believe that our search have included the majority of studies on the evaluation of ctDNA analysis for lung cancer detection. Our goal was to review the current landscape of ctDNA analysis for lung cancer detection and therefore we have summarized the ctDNA technologies used, the study design adopted and the study cohorts included in the various studies. As mentioned in the 'Discussion' section, the design of the included studies is very heterogenous, which would affect the generalizability of the studies' conclusion to clinical application. There was also high heterogeneity observed in both the cancer and control cases, which would also have implications on the interpretation of diagnostic performances and clinical adoption (e.g. complementary role with low-dose computed tomography). To conclude, our current systematic review has highlighted the number of issues in the ctDNA-based studies for lung cancer detection. We have now stated the inclusion of only 1 search engine as our study limitation.

Changes in the text: In the Discussion section, we have now stated the inclusion of only search engine as a study limitation (page 15, lines 308 – 310).

3. Methodology is overall poor.

Reply 1: We thank the reviewer for the comment. As mentioned by our previous response to Q2, we believe that our current systematic analysis have included the majority of studies and highlighted the issues of the ctDNA-based studies for lung cancer detection. Also in response to Q4, we agree that the PRISMA flowchart is incomplete and we have revised accordingly.

Changes in the text: We have revised the PRISMA as suggested in Q4.

4. Results section is vague and does not reflect their aims and endpoints. PRISMA flowchart is incomplete.

Reply 1: We thank the reviewer for the suggestion and we have revised the PRISMA flowchart, i.e. Figure 2.

Changes in the text: The PRISMA flowchart (Figure 2) is now revised.

5. Table 1 only summarises the key findings from several studies and does not construct a meaningful synthesis of data according to aims.

Reply 1: We thank the reviewer for the comment. The summary table, i.e. Table 1, was constructed to allow readers to extract the important information on the ctDNA technologies used, the study design adopted and the study cohorts (composition and the cancer and control cases) included in the various studies. Such information would allow readers to efficiently evaluate the diagnostic performances of the tested cfDNA methodologies. Also, through Table 1, the readers may appreciate the high heterogeneity of the various studies on ctDNA analysis for lung cancer detection, as mentioned in the Discussion section. This would affect the generalizability of the studies' conclusion to clinical application.

Changes in the text: (Nil)

6. A few typographic errors, and many abbreviations are not defined.

Reply 1: We thank the reviewer for the comment and we have now defined the abbreviation of DELFI as DNA evaluation of fragments for early interception in lines 243 – 244 in the manuscript. The other abbreviations in Table 1 were also defined in the table title.

Changes in the text: We have now defined the abbreviations in the Table 1.