Peer Review File

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Reviewer A

Comment 1: "At first, the authors need to have this manuscript re-written by a scientist who speaks English as a primary language. It needs considerable editing for grammar and for improving clarity at least Abstract.

Please use abbreviation correctly."

Reply 1: We have now re-written parts of the text, mainly in the abstract (see Page 2, lines 39, 40-42, 46, 56-61) in and corrected some abbreviation that were misspelled.

Comment 2: "Why is the study period "patient enrollment period" so long, 6 years? The CTC detection methods are developing and it is possible that the results will be different if the most suitable CTC detection method is used."

Reply 2: The patient enrollment period was 6 years in order to achieve a bigger sample size. We understand this may seem as a long period, however, we think this should not be considered as a bias given the type of study (prospective observational) and its aim, which is to try to elucidate the prognostic role of CTC in the perioperative setting.

Although we know that CTC detection method may be evolving, ours was based in EPCAM determination, which is the same as CELLSEARCH system (to our knowledge the only FDA approved method).

Comment 3: "Have you checked the CTC at the time of recurrence? If an increase in CTCs is demonstrated at the time of recurrence, the detection method of CTC in this study may be trusted.

In order to prove a negative study, it is necessary to prove that the measurement method is more accurate."

Reply 3: Although we have some data regarding CTC count during the patients follow up, we have not checked specifically the CTC at the time of recurrence, but it certainly would be really interesting and we will try to elaborate a new project with this purpose. Nevertheless, as we said previously, we consider our CTC detection method sufficiently accurate to prove our findings as a negative study.

Reviewer B

Comment 1: "What's the rationale for setting cut-off points as 1 and 5 CTCs for statistical

analysis? Were the value median or mean? Or is there any previous validation study for a similar cohort with surgical patients? I think this issue is critical for the meaning of this study."

Reply 1: The cut-off point of 1 was used in order to confirm the possibility of detecting CTCs in patients with early stage lung cancer, and we found that more than 75% had at least 1 detectable cell, which is similar to the results published by other groups (References 13-16). In the other hand there are some studies that consider 5 CTC as a cut-off with prognostic relevance in other solid tumors such as breast, bladder or colorectal cancer. (1–7).We have added this references to the reference list (see Page 5, line 167).

All the value were mean, as specified.

1. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin. 2017 Mar;67(2):93–9.
2. Jacob J, Krell J, Castellano L, Jiao LR, Stebbing J, Frampton AE. Determination of cut-offs for circulating tumor cell measurement in metastatic cancer. Expert Rev Anticancer Ther. 2011 Sep;11(9):1345–50.
3. Peeters DJE, Van Dam PJ, Van Den Eynden GGM, Rutten A, Wuyts H, Pouillon L, et al. Detection and prognostic significance of circulating tumour cells in patients with metastatic breast cancer according to immunohistochemical subtypes. Br J Cancer. 2014 Jan 21;110(2):375–83.
4. Müller V, Riethdorf S, Rack B, Janni W, Fasching PA, Solomayer E, et al. Prognostic impact of circulating tumor cells assessed with the CellSearch System™ and AdnaTest Breast™ in metastatic breast cancer patients: the DETECT study. Breast Cancer Res. 2012 Aug 15;14(4).
5. Krebs MG, Sloane R, Priest L, Lancashire L, Hou JM, Greystoke A, et al. Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol. 2011 Apr 20;29(12):1556–63.
6. Giuliano M, Giordano A, Jackson S, Hess KR, De Giorgi U, Mego M, et al. Circulating tumor cells as prognostic and predictive markers in metastatic breast cancer patients receiving first-line systemic treatment. Breast Cancer Res. 2011 Jun 15;13(3).
7. Cristofanilli M, Hayes DF, Budd GT, Ellis MJ, Stopeck A, Reuben JM, et al. Circulating tumor cells: a novel prognostic factor for newly diagnosed metastatic breast cancer. J Clin Oncol. 2005;23(7):1420–30.

Comment 2: “What's your rationale for this study's sample size (n=180)? A larger sample size is needed for the validation of recurrence and disease-free survival in surgical settings. I wonder if the small sample size may have produced negative results.”

Reply 2: As you said, a larger sample size may be better to validate our results. However, our study nearly triples de sample size of others such as Bayarri-Larra, C. (Reference 14) whose presurgical CTC count was not associated with OS or DFS.

Comment 3: “1. When describing abbreviations, you must describe the full word for the first. The abstract contained no descriptions for NSCLC, CTCs, DFS, OS, and MRD. And there were several undefined abbreviations in the main body.

2. On page 3, line 85, the term 'DNAct' is not familiar. I recommend replacing it with ctDNA.”

Reply 3: We have reviewed the abbreviations and corrected the ones that were misspelled or not described before.

Comment 4: “3. It would be better to add study flow, especially for blood sampling time points.

4. It would be better to show several representative figures with CTCs for the reader's understanding.

5. In Tables 3 and 4, numeric values should also be shown with percentages.”

We have added the study flow (see page 5, line 123), the CTC representative figures (see Page 6, line 160) and percentages in Tables 3 and 4 (see Pages 10 and 11).

Reviewer C

Comment 1: “1. The authors are conducting research against the background that the relationship between CTC number and prognosis in lung cancer surgery cases has not been sufficiently investigated. However, there are positive papers [1-3] and suggestive studies [4] of studies examining CTC numbers and prognosis in lung cancer surgery cases, so they should be indicated in Introduction.”

Reply 1: Thank you for your contribution, we have modified the introduction to include the references you suggested (see Page 4, line 108).

Comment 2: “Single CTC decreases in a time-dependent manner. Since the postoperative voting dates are different, bias is applied.”

Reply 2: As we emphasize in the final part of the discussion, we know that this is the main

limitation of our study, and it would be necessary to correct it in further studies.

Comment 3: “Indication and exclusion criteria are not clear.”

Reply 3: Eligible patients were those with early stage lung cancer (stages I-IIIA) that were going to be treated with surgery (see page 5, lines 118-119).

Comment 4: “4. The flow chart for case selection is not shown”.

Reply 4: We have added the flow chart (see page 5, line 123).

Comment 5: “5. The main endpoint is not clearly indicated”.

Reply 5: We have now emphasized the endpoint of the study in the introduction and material and methods (see page 5, lines 135-136).