

Peer review file

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Reviewer A

Comment 1: I see discrepancies in methodology of the study:

In the study is noted that sample “was collected in the operating room immediately before surgery” but nothing about collection after surgery. In the result and discussion is sentence: “The present study revealed that CTCs could be detected immediately after surgery for lung cancer, and that these postoperatively detected CTCs were an independent predictor of recurrence” The collection of the “postoperative blood” was after resection of the tumor? Or after lymphadenectomy? Or after skin suture?

Reply 1: We respectfully note that the original text indicated that the “postoperative CTC sample” was collected in the operating room at the completion of pulmonary resection (page 7, highlighted in yellow).

Comment 2: The postoperative detection of CTC is very dependent on the surgery technique. In the study is not specification of type surgery: open thoracotomy vs. VATS.

Reply 2: Thank you for this comment. All surgeries were performed by a single surgeon using VATS. We have indicated this in the “Surgical technique” subsection (page 6, red font).

Comment 3: Important is the order of the large vessel ligation. Standard should be priority ligation vein before artery. In study is only specification “the pulmonary vein was ligated first if possible” no more numbers or specification.

Reply 3: Vein-first ligation was performed in 36 cases (92.3%) that involved lobectomy. This information has been added on page 6 (red font).

Comment 4: Secondly the shedding of the CTC in cancers is very uneven and depend on the surgeon and time of surgery. These data are missing.

Reply 4: Thank you for this comment. The operation time results were added to Table 1 (red font) and we have indicated that a single surgeon performed the procedures using

VATS (page 6, red font).

Comment 5: The level of CTC was examined only from 3 mL of blood, it is very small quantity. Other method (not only size based) using minimal 8 ml for CTC detection. For evaluation of patient is need more than only one preoperative collection of blood. Optimal should be minimal 2 or 3 collections in different time.

Reply 5: While we agree with your comment, the present study did not aim to compare CTC detection methods, but rather to determine the clinical relevance of CTC detection immediately after lung cancer resection in cases without preoperatively detected CTCs. We have noted that the blood sample size might be a limitation (page 13, red font). We hope to perform additional studies to incorporate larger samples and different methods for postoperative CTC detection.

Comment 6: I see the problematic methodology in this study. I am sure that in the study were enrolled false negative patients. The number of patients is very small in each group. IN this situation is not possible to take conclusion about prediction “postoperatively detected CTCs were an independent predictor of recurrence”.

Reply 6: Thank you for this comment. While we agree that this study does not provide the level of evidence that a properly-sized RCT would, all studies and settings have some limitations. We have revised the Discussion to include a more detailed explanation of the study’s limitations (page 13, red font).

Reviewer B

This is an interesting piece of work especially with the role of CTCs being critical in understanding the tumor biology in real-time. Lung cancer being the leading cause of cancer-related mortality deserves more in-depth investigations with respect to CTCs.

Important findings and strengths of the manuscript

1. The role of surgical manipulations in 'CTC-spillage' into circulation
2. The role of post-operative CTCs in disease-free and overall survival
3. Prognostic roles of CTCs and CTC clusters in disease outcome
4. Experimental findings are supported by extensive clinical findings/data

In spite of important findings, the manuscript lacks several important details.

Comment 1: Type of lung cancer (small cell or non-small cell lung cancer)

Reply 1: We apologize for this oversight and have revised the title (page 1, red font), abstract (page 3, red font), and text to indicate that the cases involved histologically confirmed non-small cell lung cancer.

Comment 2: Method used for detecting pulmonary nodule(s).

Reply 2: All pulmonary nodules were evaluated using computed tomography. We have revised the “Patient selection” subsection to address this point (page 6, red font).

Comment 3: Authors have mentioned enrichment methodology; but it is highly appropriate to provide details of staining method (antibodies used) to determine the cytological markers expressed.

Reply 3: While we agree that immunostaining methods are an important consideration, although epithelial markers might not be detected for “cluster CTCs”, which could provide a false result based on immunostaining alone. Thus, we believe it is important to also perform a morphological evaluation to identify cluster CTCs. We have revised the Discussion section to address this point (page 12, red font).

Comment 4: Volume of blood used for CTC detection.

Reply 4: We agree that a larger blood sample volume might increase sensitivity. We have mentioned this as a study limitation in the Discussion (page 13, red font).

Comment 5: Average time between blood collection and blood processing for CTC enrichment.

Reply 5: Unfortunately, we did not record the intervals between the blood collection, blood processing, and CTC enrichment. However, all samples were evaluated within 1 h after the end of the operation. We have revised the “Detection of CTCs” subsection to address this point (page 7, red font).

Clarifications needed

Comment 6: Some set of patients had only single CTCs which make total sense.

Reply 6: It seems logical that postoperative single CTC detection would predict recurrence.

We have revised the Discussion to address this point (page 12, red font).

Comment 7: Some set of patients had CTC clusters. But it is important to provide whether these patients with CTC clusters had individual CTCs also. Please check and justify your findings.

Reply 7: Thank you for this comment. All patients with cluster CTCs also had single CTCs. We have revised the Results section to address this point (page 9, red font).