Peer Review File

Reviewer A

Intraoperative wedge Resektion before Lobektomie for onkological purposes is an important message, that we have all been anticipating. I am glad that You have found a way to quantify the relative tumor cell load after surgery. I congratulate You to a splendid work.

Reply 1: Thank you very much for your comments and positive affirmation.

Reviewer B

Actually i have several points regarding the study design and the study content.

1- STUDY DESIGN : The study includes 2 different cohorts , The main one is several hunders and the other is only tens of patients. This sounds very strange to include two diffrent cohorts in the same study without direct relation.

The patients group of FR+CTC should be in a separate study (probably a pilot study).

Reply 1: Thank you very much for your comments. Our research is designed to imitate an article[1]. The survival outcomes were not available in CTC study because of an insufficient follow-up period. Thus, we conducted a retrospective analysis of a lung cancer registry using the same inclusion and exclusion criteria to evaluate the survival outcomes. The patients group of FR+CTC is a pilot study. Further prospective studies are warranted.

Changes in the text:

2- PATIENT AND METHOD:

A- What were indications to wdge+ lobectomy and why was the number of this group nearly double the number of lobectomy group?

Reply 2: Our selection criteria: a) patients who received standardized video-assisted thoracic surgery (VATS) lobectomy; b) pathologically confirmed NSCLC with stage

T1N0M0 that determined by two experienced pathologists after surgery; c) had peripheral tumor suitable for wedge resection of the lung; d) had Karnofsky performance score ≥70. Exclusion criteria were a) no systemic lymph node dissections during surgery. b) purely ground glass nodules (GGO). c) pathological diagnosis of adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA). d) absence of information on extracted data. Therefore, all the cases we included can be wedge resection of the tumor first. Since most lung tumors have no pathological diagnosis before surgery, more surgeons will choose to perform wedge resection first. However, not all doctors will choose to perform wedge resection for peripheral nodules due to different surgical habits. First, the imaging of lung tumors considers the possibility of malignancy is extremely high, and direct lobectomy can shorten the operation time and reduce the cost. Secondly, some patients have been diagnosed with preoperative pathology. Moreover, in some patients, the lung tumor is deeper and larger, and it may be difficult to perform lung wedge resection.

Changes in the text: Since most lung tumors have no pathological diagnosis before surgery, more surgeons will choose to perform wedge resection first. However, some doctors will consider direct lobectomy due to the following possible reasons: First, the imaging of lung tumors considers the possibility of malignancy is extremely high so that direct lobectomy can shorten the operation time and reduce the cost. Secondly, some patients have been diagnosed with preoperative pathology. Moreover, the lung tumor is deeper and larger in some patients so that it may be difficult to perform lung wedge resection.(line 240-247)

Patients were included if they met the following conditions: a) patients who received standardized video-assisted thoracic surgery (VATS) lobectomy; b) pathologically confirmed NSCLC with stage T1N0M0 that determined by two experienced pathologists after surgery; c) had peripheral tumor suitable for wedge resection of the lung; d) had Karnofsky performance score ≥70. Patients not meeting the inclusion criteria were excluded. Other exclusion criteria were as follows: a) no systemic lymph node dissections during surgery. b) purely ground glass nodules (GGO). c) pathological diagnosis of adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma

(MIA). d) absence of information on extracted data. The TNM staging system was characterized according to the eighth edition of the American Joint Committee on Cancer (AJCC).(line 116-126)

B- The study did not take in consideration co-morbidities like other malignancies which are very crucial in studies investigating overall survival in elderly patients.

Reply 3: Thank you very much for your comment. Co-morbidities like other malignancies which are very crucial in studies investigating overall survival in elderly patients. This is a limitation for our study.

Changes in the text:

C- In the statistical analysis, you include death in the definition of DFS. Yes,Did you exclude the death other than disease burden? otherwise the statistics would be uncertain.

Reply 4: Thank you very much for your comment. The DFS has excluded the death other than disease burden.

Changes in the text: Disease-free survival (DFS) was defined as the time from surgery to the date of tumor progression or death (from cancer) or last follow-up.(line178-189)

3- Linguistic flaws: I would like to point some notes about english words, terms, contractions and phrases.

Examples;

- (Web +Lob) or (Wed +Lob) row 197, 198 and 201.

- Row 181 (that no between-group.....)

- Row 129 after d) the letter is e) not f), and it is better to write a-, b- and so on.

- Row 270 check the spelling and the construction of the meaning.

Reply 4: I am very sorry for the mistakes. These errors have been corrected, and the English grammar and syntax of the revised manuscript have been corrected by a native English-speaking editor.

Changes in the text: Wed +Lob (line 190-192); Survival analysis by log-rank tests showed that no difference between the groups in OS;(line 201) e) preoperative and postoperative FR+CTCs results;(line144) For example, more patients with larger and deeper lung nodules chose direct lobectomy.(line 297-298)

The authors demonstrated the impact of wedge resection just before the surgery. However, their statistical analysis must be strange. In this study, All patients are Stage I lung cancer, therefore the tumor size should be matched with the threshold of 3cm. Web+Lob group contains less Stage IB lung cancer and more Stage IA3 than Lob group, which might lead to the difference in OS.

Reply 1: Thank you very much for your comment. Our research object is T1N0M0 patients, so there will be no Stage IB lung cancer patients according to the eighth edition staging. Moreover, a propensity score matching analysis was performed to reduce the effects of selection bias. There is no significant difference between the two groups in tumor size, so the number of the two groups has no significant difference in IA1 IA2 and IA3 stages.

Changes in the text:

The authors reported of prognostic significance of wedge resection of tumor before lobectomy in patients with NSCLC and additional exploratory research using CTCs.

I have some questions and arguments to interpret their results. We request the authors corrections or additional explanations.

Major comments:

1. To begin with, the authors should clearly state about their indication of wedge resection before lobectomy. Although they included 813 patients who had peripheral disease which is able to perform wedge resection, 282 patients did not perform it. Why?

Were their diseases pathologically diagnosed before surgery? Is there any possibility that they had deeper lesions which were challenging to perform wedge resection?

Reply 1: Thank you very much for your comments. Our selection criteria:a) patients who received standardized video-assisted thoracic surgery (VATS) lobectomy; b) pathologically confirmed NSCLC with stage T1N0M0 that determined by two experienced pathologists after surgery; c) had peripheral tumor suitable for wedge resection of the lung; d) had Karnofsky performance score ≥70. exclusion criteria were a) no systemic lymph node dissections during surgery. b) purely ground glass nodules (GGO). c) pathological diagnosis of adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA). d) absence of information on extracted data. Therefore, all the cases we included can be wedge resection of the tumor first. Since most lung tumors have no pathological diagnosis before surgery, more surgeons will choose to perform wedge resection first. However, not all doctors will choose to perform wedge resection for peripheral nodules due to different surgical habits. First, the imaging of lung tumors considers the possibility of malignancy is extremely high, and direct lobectomy can shorten the operation time and reduce the cost. Secondly, some patients have been diagnosed with preoperative pathology. Moreover, in some patients, the lung tumor is deeper and larger, and it may be difficult to perform lung wedge resection.

Changes in the text: Since most lung tumors have no pathological diagnosis before surgery, more surgeons will choose to perform wedge resection first. However, some doctors will consider direct lobectomy due to the following possible reasons: First, the imaging of lung tumors considers the possibility of malignancy is extremely high so that direct lobectomy can shorten the operation time and reduce the cost. Secondly, some patients have been diagnosed with preoperative pathology. Moreover, the lung tumor is deeper and larger in some patients so that it may be difficult to perform lung wedge resection.(line 240-247)

Patients were included if they met the following conditions: a) patients who received standardized video-assisted thoracic surgery (VATS) lobectomy; b) pathologically confirmed NSCLC with stage T1N0M0 that determined by two experienced

pathologists after surgery; c) had peripheral tumor suitable for wedge resection of the lung; d) had Karnofsky performance score ≥70. Patients not meeting the inclusion criteria were excluded. Other exclusion criteria were as follows: a) no systemic lymph node dissections during surgery. b) purely ground glass nodules (GGO). c) pathological diagnosis of adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA). d) absence of information on extracted data. The TNM staging system was characterized according to the eighth edition of the American Joint Committee on Cancer (AJCC).

2. I feel it is difficult to conclude that wedge resection before lobectomy contribute to improve DFS through preventing the tumor cell spread during surgery from the results of this study.

The authors should make an effort to rule out the possibility that the results were just a coincidence.

Is there a need to include pathological factors, such as lymphovascular involvement, pleural invasion and adenocarcinoma subtype in the propensity score matching?

To exclude the effect of other-cause death on survival analysis, Presenting the cumulative recurrence rate of the two group would be also a good idea.

Reply 2: Thank you very much for your suggestions. Some pathological factors, such as lymphovascular involvement, pleural invasion and adenocarcinoma subtype has been ruled out in our case. Because our research object is T1N0M0 patients and it excluded patients with purely GGO, AIS and MIA.

Disease-free survival (DFS) was defined as the time from surgery to the date of tumor progression or death (from cancer) or last follow-up. We have excluded the effect of other-cause death on survival analysis. The cumulative recurrence rate is indeed a good indicator. However, since the current original data cannot be restored to each specific patient, this data cannot be recollected.

Changes in the text: Disease-free survival (DFS) was defined as the time from surgery to the date of tumor progression or death (from cancer) or last follow-up.(line78-79)

3. The authors included the patients with T1N0M0 disease. Did this study comply with the 8th edition of TNM classification? They should clearly describe about it in Patients and Methods.

In Table 1, What the meaning of tumor size? Does it mean clinical size or pathological size? If pathological size, total tumor size or invasive component size? The authors need to explain in detail because this is one of the important points.

Reply 3: Thank you very much for your suggestions. The TNM staging system was characterized according to the eighth edition of the American Joint Committee on Cancer (AJCC).

This is the largest diameter of the tumor on CT imaging. The pathological size is difficult to measure to the maximum diameter of the tumor, and the size of the tumor soaked in formalin may be affected. And we excluded patients with purely ground glass nodules (GGO), AIS and MIA.

Changes in the text: The TNM staging system was characterized according to the eighth edition of the American Joint Committee on Cancer (AJCC). (line125-126)

4. Table 2 and Figure 1 do not consistent. For example, In Table 2, 5-year DFS of Lob was 88.6%, however, according to KM curve of Figure 1B, DFS rate was under 80% at 60 months.

In addition, the author should state that the population in Table or Figure is the matched cohort, aside from the manuscript.

Reply 4: Thank you very much for your comments. I am very sorry for the mistakes in table 2. The data in the table has been completed and carefully checked. Thank you for your suggestion to state that the population in Table or Figure is the matched cohort.

Changes in the text:

Table 2. OS rate and DFS rate in the matched patients with T1N0M0 non-small cell lung cancer		
Wed+Lob	Lob	P

OS rate (%)			0.17
1year	99.2%	98.8%	
3year	98.4%	95.0%	
5year	89.9%	88.4%	
DFS rate (%)			0.006
1year	98.7%	94.0%	
3year	90.6%	84.5%	
5year	82.2%	74.0%	

Abbreviations: OS, Overall survival; DFS, Disease-free survival. Lob: Direct lobectomy; Wed+Lob: Wedge resection of tumor followed by lobectomy

Fig 1. Kaplan-Meier estimates of the surgical approach and overall survival time and disease-free survival in the matched patients with T1N0M0 non–small-cell lung cancer.

Table S1 Multivariable Analysis of OS and DFS in the matched Patients with T1N0M0 Non-Small Cell Lung Cancer.

Minor comments:

1. The authors should avoid using abbreviation, FR+CTCs in the first statement in page 7, aside from abstract.

Reply 1: Thank you very much for your suggestions.

Changes in the text: We conducted an exploratory study to investigate folate receptor–positive circulating tumor cells (FR+CTCs) levels at 'Blinded per Author Guidelines' hospital from September 15, 2018, to April 15, 2020.(line 137-138)

2. In Figure 2, preoperative CTC levels were apparently high in Lob group. Please explain about the difference.

Reply 2: Thank you very much for your comments. Preoperative CTC levels were apparently high in Lob group because the Y-axis measurement unit is different in the

two figures. In fact, there was no significant change in the Lob group VS Wed + Lob group before surgery (14.0 (±5.9) FU per 3 mL vs. 16.6 (±9.4) FU per 3 mL, $P = 0.281$). Changes in the text: In the Wed+Lob group, FR+CTCs levels before surgery were significantly higher than those after surgery (16.6 (±9.4) FU per 3 mL vs. 10.5 (±5.7) FU per 3 mL, $P = 0.011$; Fig. 2A). In the Lob group, no significant changes in FR+CTCs were observed before and after surgery (14.0 (±5.9) FU per 3 mL vs. 12.7 (±5.4) FU per 3 mL, $P = 0.44$; Fig. 2B) (line219-222)

3. In Table 2, the number of Age >70 years in Web+Lob group after matching (74) should be corrected (2).

Reply 3: Thank you very much for your suggestions. I am very sorry for the mistakes in table.

Changes in the text:

Table 3 Baseline characteristics before and after propensity score matching for CTC cohort						
	Before matching (n=62)			After matching (n=46)		
	Wed+Lob (n=33)	Lob (n=29)	*P*-value	Wed+Lob (n=23)	Lob (n=23)	*P*-value
Age			0.201			0.525
<60	15 (45.5%)	17 (58.6%)		11 (47.8%)	12 (52.2%)	
60-70	16 (48.5%)	8 (27.6%)		11 (47.8%)	8 (34.8%)	
≥70	2 (6.1%)	4 (13.8%)		1 (4.3%)	3 (13.0%)	

References

[1]   Wei S, Guo C, He J, et al. Effect of Vein-First vs Artery-First Surgical Technique on Circulating Tumor Cells and Survival in Patients With Non-Small Cell Lung Cancer: A Randomized Clinical Trial and Registry-Based Propensity Score Matching Analysis. JAMA Surg. 2019. 154(7): e190972.