Peer Review File

Reviewer #1:

1. This is an original retrospective study that evaluates the effect of pathological factors and preoperative smoking status and history on post-operative survival in patients with stage I NSCLC who underwent complete resection and systematic lymph node dissection at a single center in Japan. A relatively large group of patients is analysed (n=453). The main conclusions are that preoperative smoking status and history promote tumor progression, particularly in adenocarcinomas ≤3 cm, and that a period of at least 10 years of smoking cessation would be associated with postoperative survival benefits. In the opinion of this reviewer, the research does not provide novel findings and presents concerns in the study methods.

Reply 1: Thank you for giving us the opportunity to explain the novelty of our findings. In this study, we attempted to answer two clinical questions regarding preoperative smoking.

A. What is the influence of preoperative smoking on the progression and invasiveness of stage I NSCLC?

In the current TNM staging, particularly in lung adenocarcinomas, there is a modification in tumor size measurement. Accordingly, the invasive size instead of whole tumor size is to be described in the clinical/pathological report, as it reflects tumor invasiveness more precisely. However, even though invasive size is same, progression and invasiveness of early stage lung adenocarcinoma in smokers and non-smokers may be different. For answering this question, we compared stage I NSCLC in smokers and non-smokers, evaluating the frequency of NSCLC with invasive factors such as vascular invasion and pleural invasion. To help readers understand easily, we have added Table 3 that shows the relationship between pathological factors and smoking status.

B. How long should they have been smoke-free prior to the diagnosis of NSCLC if they want to achieve a postoperative prognosis similar to that of non-smokers?

The numbers of smokers who quit smoking are increasing recently due to the worldwide educational efforts regarding the dangers of smoking. Unfortunately, some ex-smokers suffer from NSCLC. We frequently meet lung cancer patients with tobacco history. They sometimes ask us when they should have quit smoking. We cannot tell them the definitive years that would make up for the past smoking history in terms of postoperative survival. But we want to inform them the duration of smoking cessation that can provide patients with NSCLC and smoking history similar postoperative prognosis as that of non-smokers. That would potentially encourage current smokers to quit smoking.

To emphasize these points, we have added the following sentences in the revised manuscript.

Changes in the text:

Additionally, there is an increase in the number of smokers who quit smoking owing to the worldwide

educational efforts regarding the importance of smoking cessation as a means of preventing lung as well as other cancers (8, 13). Unfortunately, some ex-smokers may still be diagnosed with NSCLC. In order to encourage current smokers to quit smoking, we should be able to provide information regarding the duration of smoking cessation prior to the diagnosis of stage I NSCLC that may result in outcomes and prognosis in smokers similar to that in non-smokers. However, this information is currently unavailable. (page 5, lines 58-63)

Relationship between pathological factors and smoking status

The statistical relationship between clinical and pathological factors among patients with adenocarcinoma and non-adenocarcinoma are presented in Table 3. While not only invasive tumor size but also tobacco habit was significantly correlated with pathological factors among adenocarcinoma patients, there was no such relationship in patients with non-adenocarcinomas. These results indicate that smoking may contribute to the progression of adenocarcinoma. (pages 9-10, lines 125-130)

Ideally, all smokers should quit smoking for the prevention of NSCLC. Although there is a reduction in the numbers of smokers worldwide, several still love smoking (21). There is inadequate information regarding the duration of smoking cessation prior to the diagnosis of stage I NSCLC that may result in outcomes and prognosis in smokers similar to that in non-smokers. Such information, if available, may be useful in encouraging smokers to quit. (page 13, lines 193-196)

2. The study lacks a multivariate analysis which takes into account important confounding factors, for example, age at the lung cancer diagnosis, sex, comorbidity.

Reply 2: Thank you for your comment. We agree on your suggestion that multivariate analysis would add to the data provided. In the revised submission, we have included multivariate survival analyses as shown in Table 2. Vascular invasion and pleural invasion were identified as independent prognostic factors among adenocarcinoma patients on multivariate analyses. Please note that we do not underline that smoking habit is a prognostic factor in resected NSCLC in this table. It is our intention to inform readers that these pathological variables are not only prognostic factors but also potential surrogate markers of tumor malignancy.

3. The study has important limits, as also acknowledged by the authors, in particular, the lack of information on the post-operative smoking status or the use of post-operative treatments which may have affected the survival.

Reply 3: Thank you for your comment. We agree with you that postoperative smoking status may have an important influence on postoperative survival. We rely on patient's affirmation when we ask lung cancer patients with past smoking history whether they can continue abstinence from smoking following their lung resection. Almost all patients tell us "yes." Actually, we do not ask the same question in every follow-up clinic. Ideally, we should check their postoperative smoking status using objective measurements such as carboxyhemoglobin

and cotinine. But, since this is a retrospective study, we could only record the patients' responses. In terms of post-operative treatment, at our institute, we recommend p-stage IB NSCLC patients for postoperative adjuvant chemotherapy. After recurrence is confirmed, treatment differs depending on the recurrence pattern and genetic status of tumors. We have made the following modifications in our revised manuscript:

Changes in the text:

This study has some limitations. First, this was a retrospective study at single Institute; therefore, patient selection bias and time trend bias are inevitable. Second, we did not include the postoperative treatments such as adjuvant therapy and post-recurrence treatment in our analyses. Genetic differences may influence these treatment modalities, which could prolong survival after surgery and relapse. Third, we did not focus on postoperative smoking status because we did not ask patients if they had resumed smoking following surgery in every follow-up clinic. We also did not collect information regarding second-hand smoking. Fourth, our analyses regarding smoking history were based on medical records. Objective measurements such as carboxyhemoglobin and cotinine may have been more reliable biomarkers to evaluate tobacco smoking exposure. Fifth, in the analyses of non-adenocarcinomas, it is possible that true probability may not have been obtained owing to the small sample size. Additionally, postoperative survival for each segment of smoking cessation period should be compared to confirm the number of years after cessation that can improve the postoperative survival (e.g., never vs. 5 y.c. vs. 10 y.c. vs. 15 y.c. vs. 20 y.c.). Further investigations with a larger number of patients are required to substantiate our findings. (page 15, lines 221-224)

4. The main variable of outcomes (i.e. overall survival, recurrence-free survival, disease-specific survival) may be affected by a bias of definition (see Follow-up assessments, lines 97-102); indeed, for each of the considered outcomes, it should be reported the proportion of the subjects actually dead or lost-to-follow-up.

Reply 4: Thank you for your suggestion. Among 325 adenocarcinoma patients, 49 patients (15.1%) died and 49 (15.1%) patients had recurrent tumor. In 128 non-adenocarcinoma patients, 34 (26.6%) died and 23 cases (18.0%) relapsed. Patients with a follow-up of less than one year were excluded. Among living patients, 57.2% of adenocarcinoma patients and 61.7% of non-adenocarcinoma patients had an incomplete observation period (within 5 years). The mean observation period of all living patients was 58.7 months, which is almost 5 years, as shown in Table 1. As a reference, we described the transition of patient numbers as "patients at risk" in Figure 2 and 3.

Changes in the text:

Overall, 453 patients with pathological stage I NSCLC who underwent complete resection and systematic lymph node dissection at our Institute between January 2004 and December 2018 were enrolled in this study. The cases with invasive diameter > 4 cm or lymph node metastasis were excluded based on pathological examinations. Segmentectomy with systematic lymphadenectomy was the procedure included as limited surgery in our retrospective study. Patients who underwent wedge resection and/or neoadjuvant chemotherapy were excluded.

Patients with a follow-up of less than one year were excluded (Table 1, Figure 1). (page 6, lines 79-80)

Pathological factors that would promote tumor progression in pathological stage I NSCLC

In the survival analyses of 453 patients, median survival period was 137 months (95% confidence interval, 130-144 months). Five- and 10-year survival rates were 84.9% and 68.4% for OS, and 75.8% and 62.6% for RFS, respectively. Among 325 adenocarcinoma patients, 49 patients (15.1%) died and 49 (15.1%) patients had recurrent tumors. In 128 non-adenocarcinoma patients, 34 (26.6%) died and 23 cases (18.0%) relapsed. (page 9, lines 112-117)

5. Number of years of smoking and pack-years are variables intracorrelated (years of smoking is used for the calculation of the pack-years). Indeed, both these variables provide similar results in table 3, 4, and 6.

Reply 5: Thank you for your comment. The various measurements to evaluate smoking status are well known. We used 4 measurements including cigarettes per day, smoking years, pack-years, and smoking cessation years. As mentioned in the discussion, since there is no consensus regarding the best method for smoking evaluation, we decided to measure these four parameters to study whether smoking has a strong association with local invasion and lung cancer progression

We also examined the number of cigarettes per day, smoking years, and smoking cessation years. Similar tendencies regarding the relationships with pathological factors were observed (Tables 4, 5, and 7). Although we cannot comment on the evaluation method best suitable to predict postoperative survival in these patients, smoking is certainly associated with local invasion and progression of lung adenocarcinomas. (pages 14-15, lines 213-215)

6. The conclusions in the abstract and in the main text should be made uniform.

Reply 6: Thank you very much for your comment. We have accordingly made the following correction:

Changes in the text:
Conclusions: Accumulative smoking habit correlated with VI and PL, particularly in 2–3 cm adenocarcinoma, whereas larger adenocarcinomas and non-adenocarcinomas of any size appear to grow and become invasive independent of preoperative smoking status. Longer smoking cessation ≥ 10 years can result in postoperative survival similar to that of non-smokers with adenocarcinomas ≤3 cm. Current smokers should quit smoking immediately to ensure longer survival even thoughthey suffer from small-sized lung adenocarcinomas in the future. (page 3, lines 37-39)

Smoking promotes tumor progression, which is represented by VI and PL, particularly in pathological stage I

adenocarcinomas measuring ==2–3 cm.== ==Larger adenocarcinomas and non-adenocarcinomas of any size may grow== ==and become invasive independent of the preoperative smoking status.== Ten or more years of smoking cessation may have associated postoperative survival benefits. Outcomes appear to be proportionally related to the duration of smoking cessation. Therefore, ==current smokers should quit smoking to ensure longer survival even== ==though they suffer from small-sized lung adenocarcinomas in the future==. (page 16, lines 230-235)

7. Abstract, lines 27-28, and Introduction, lines 70-71. The expression ".. period of smoking cessation that would "cancel" the past smoking history .." should be changed. The used term implies a condition that cannot be obtained, which is the complete elimination of the risk of death for lung cancer due to lifetime smoking exposure.

Reply 7: Thank you for your suggestion. We agree with you that "cancel" is not a suitable verb to express the intended meaning. We have replaced this word with "compensate" or "make up for" as a better alternative in the revised manuscript.

Changes in the text:
This study aimed to investigate the period of smoking cessation that may "==compensate==" for past smoking history regarding postoperative survival in cases of resected ==pathological== stage I NSCLC by examining the relationship between clinicopathological factors and preoperative smoking. (page 2, line 22)

In this study, we examined the relationship between prognostic factors and preoperative smoking status in cases of resected ==pathological== stage I NSCLC. We also investigated the period of smoking cessation that would ==make== ==up for== the past smoking history in terms of postoperative survival. (page 5, line 66)

8. Introduction, lines 62-64. A reference should be quoted for this affirmation. Indeed, the risk of developing adenocarcinoma of the lung from cigarette smoking has increased since the 1960s (see for example the "The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. 2014").

Reply 8: Thank you for your suggestion. Accordingly, we have added references as well as modified the text in the revised manuscript.

Changes in the text:
Non-adenocarcinomas, such as squamous cell carcinomas, are generally considered to be associated with heavy smoking ==(8). Low yield filter cigarettes with deeper and more frequent inhalation may result in increased== ==delivery of carcinogens to the lung peripheries. This may contribute to an increased incidence of== ==adenocarcinoma of the lung (9)==. Non-smokers with specific genetic features too can develop lung adenocarcinoma ==(10)==. (page 4, lines 52-55)

References

8.   Wynder EL, Muscat JE. The changing epidemiology of smoking and lung cancer histology. Environ Health Perspect 1995;103(Suppl8):143-148.

9.   Nakamura H, Saji H. Worldwide trend of increasing primary adenocarcinoma of the lung. Surg Today 2014;44(6):1004-1012.

10.   Stellman SD, Muscat JE, Thompson S, Hoffmann D, Wynder EL et al. Risk of squamous cell carcinoma and adenocarcinoma of the lung in relation to lifetime filter cigarette smoking. Cancer 1997;80(3):382-388.
(page 21, lines 273-285)

9. Tables 3, 4, 6. Substitute: "Per day" with "Cigarettes currently smoked per day"; "Years" with "Years of smoking"; "Pack year" with "Pack-years". Provide unit of measurements (i.e. number).

10. Tables 3, 4, 6. Check data for the column "Pack year", see for example "515" in the first row of table 3.

11. Explain the abbreviation "y.c.".

Reply 9-11: Thank you for these comments. Based on your advice, we have corrected the concerned tables (New Table 4, 5, 7).

Reviewer #2:

1. The study question is relevant, but the results should be interpreted with caution as this is a retrospective study extending over a long-time period during which diagnostic, staging and therapeutic algorithms changed significantly

Reply 1: Thank you for your comment. As rightly pointed out by you, our study has a few important limitations, especially owing to its retrospective design. For pathological staging, all histopathology slides were reviewed and re-evaluated according to the current 8th TNM edition.

This study has some limitations. First, this was a retrospective study at single Institute; therefore, patient selection bias and time trend bias are inevitable. Second, we did not include the postoperative treatments such as adjuvant therapy and post-recurrence treatment in our analyses. Genetic differences may influence these treatment modalities, which could prolong survival after surgery and relapse. (page 15, lines 216-219)

2. The authors refer to stage I: was this clinical or pathological stage I NSCLC? What was the concordance between both?

Reply 2: Thank you for raising this important question. All cases were pathologically-proven stage I NSCLC. We appreciate your comment. We have added "pathological" to stage I in a few sentences as well as to the

manuscript title. The concordance rate between c- and p-stage I was 89.6%.

Changes in the text:

Title: How long is cessation of preoperative smoking required to improve postoperative survival of patients with ==pathological== stage I non-small cell lung cancer?

3. Line 80: quite a lot of patients were excluded; the authors should construct a GANTT chart with the total number of operated patients between 2004 and 2018 and then list all excluded cases with the specific reason for it, and finally end with the number of included patients

Reply 3: Thank you for your comment. Since we are not very familiar with a GANNT chart, we added a study flow chart as Figure 1 to help to clarify the reasons for exclusion.

4. The authors should indicate how the smoking status was recorded: by history only? by whom (nurse, medical student, fellow)? any objective measurements as carboxyhemoglobin or cotinine which is the best biomarker of tobacco smoke exposure? This is important as many patients are not quite honest when asked about their smoking status
5. Unfortunately, postoperative smoking status was not fully recorded (line 204), this represents a confounding factor as patients who continue or resume smoking have a poorer prognosis, also due to their comorbidity

Reply 4, 5: Thank you for your comment. Data of smoking history was collected from medical records by nurses and/or doctors. As pointed out by you, postoperative smoking status may have an important influence on postoperative survival. We rely on patient's affirmation when we ask lung cancer patients with past smoking history whether they can continue abstinence from smoking following their lung resection. Almost all patients tell us "yes." Actually, we do not ask the same question in every follow-up clinic. Ideally, we should check their postoperative smoking status using objective measurements such as carboxyhemoglobin and cotinine. But, because this is a retrospective study, we could only record their answers. We modified our methods and limitations to reflect these clarifications. Regarding comorbidity, COPD was included in all survival analyses in this revised manuscript. Also, disease-specific survival was analyzed to eliminate death that is not directly related to tumor relapse but to conditions such as coronary disease.

Changes in the text:

This study was approved by our institutional review board in March 2020 (approved number # 2483). The requirement of informed consent was waived due to the retrospective design of the study and because no personally identifiable information was collected. ==Clinical data including smoking history were recorded by medical staff (nurses and/or doctors) and used for statistical analysis.== (page 6, lines 79-80)

Third, we did not focus on postoperative smoking status because we did not ask patients if they had resumed

smoking following surgery in every follow-up clinic. We also did not collect information regarding second-hand smoking. Fourth, our analyses regarding smoking history were based on medical records. Objective measurements such as carboxyhemoglobin and cotinine may have been more reliable biomarkers to evaluate tobacco smoking exposure. Fifth, in the analyses of non-adenocarcinomas, it is possible that true probability may not have been obtained owing to the small sample size. (page 15, lines 221-224)

6. The comorbidity of smoking patients is equally important and also related to long-term survival: how many patients had COPD, coronary ischemic heart disease? How was comorbidity related to outcome?

Reply 6: Thank you for your comment. Among 453 patients, 103 were diagnosed to have COPD before surgery. Since almost all COPDs were mild (stage I according to GOLD staging), COPD was not identified as a prognostic factor in survival analyses as shown in Table 2. We added COPD data to the other tables. Additionally, 58 patients had history of treatment for coronary disease. We always ask cardiologists if such patient can tolerate lung resection. Based on medical records, 3 patients died of cardiovascular disease (AMI, heart failure).

7. Regarding statistical analysis, a multivariate analysis should be performed to determine whether smoking status was an independent prognostic factor or not

Reply 7: Thank you for your comment. We agree on your suggestion that multivariate analysis would add to the data provided. In the revised submission, we have included multivariate survival analyses as shown in Table 2. Vascular invasion and pleural invasion were identified as independent prognostic factors among adenocarcinoma patients on multivariate analyses. Please note that we do not underline that smoking habit is a prognostic factor in resected NSCLC in this table. It is our intention to inform readers these pathological variables are not only prognostic factors but also potential surrogate markers of tumor malignancy.

8. Some absolute survival data should be provided: median survival, 5- and 10-year overall and disease-free survival rates (with 95% confidence interval)

Reply 8: Thank you for your comment. Median survival was 137 months (130-144). 5- and 10-year survival rates were 84.9% and 68.4% for OS and 75.8% and 62.6% for RFS, respectively. We added this information in the revised manuscript.

Changes in the text:

Pathological factors that would promote tumor progression in pathological stage I NSCLC

In the survival analyses of 453 patients, median survival period was 137 months (95% confidence interval, 130-144 months). Five- and 10-year survival rates were 84.9% and 68.4% for OS, and 75.8% and 62.6% for RFS, respectively. Among 325 adenocarcinoma patients, 49 patients (15.1%) died and 49 (15.1%) patients had

(page 9, lines 112-117)

9. Do the authors have any data on specific mutations as EGFR, ROS, ALK in their patient population as these may also determine the long-term outcome, especially in an Asian population

Reply 9: Thank you for your comment. Because we did not analyze genetic information of resected tumors in cases without tumor recurrence, we did not have that information for all cases. We mentioned this in our discussion as follows:

Second, we did not include the postoperative treatments such as adjuvant therapy and post-recurrence treatment in our analyses. Genetic differences may influence these treatment modalities, which could prolong survival after surgery and relapse. (page 15, lines 217-219)

10. What were the causes of death? were recurrences mainly distant or locoregional?

Reply 10: Thank you for your comment. Among 453 patients, 83 patients died, and 72 patients had recurrent tumors (some cases have an overlap). The causes of death were tumor relapse (45), pulmonary disease including pneumonia and acute exacerbation of IPF (15), another type malignancy including leukemia (10), cardiovascular disease including AMI and heart failure (3), and cerebral vascular disease (2). The cause of death in 10 other cases were not clear from their medical records. In terms of recurrence patterns, distant/locoregional relapse was seen in the ratio of 45/22. Five patients had multiple recurrences (both distant and locoregional relapse).

11. What are the clinical implications of this study? do the authors suggest adjuvant therapy in some high-risk groups?

Reply 11:
Thank you for giving us the opportunity to explain the novelty of our findings. In this study, we attempted to answer two clinical questions regarding preoperative smoking.

A. What is the influence of preoperative smoking on the progression and invasiveness of stage I NSCLC?
In the current TNM staging, particularly in lung adenocarcinomas, there is a modification in tumor size measurement. Accordingly, the invasive size instead of whole tumor size is to be described in the clinical/pathological report, as it reflects tumor invasiveness more precisely. However, even though invasive size is same, progression and invasiveness of early stage lung adenocarcinoma in smokers and non-smokers may be different. For answering this question, we compared stage I NSCLC in smokers and non-smokers, evaluating the frequency of NSCLC with invasive factors such as vascular invasion and pleural invasion. To

help readers understand easily, we have added Table 3 that shows the relationship between pathological factors and smoking status.

B. How long should they have been smoke-free prior to the diagnosis of NSCLC if they want to achieve a postoperative prognosis similar to that of non-smokers?

The numbers of smokers who quit smoking are increasing recently due to the worldwide educational efforts regarding the dangers of smoking. Unfortunately, some ex-smokers suffer from NSCLC. We frequently meet lung cancer patients with tobacco history. They sometimes ask us when they should have quit smoking. We cannot tell them the definitive years that would make up for the past smoking history in terms of postoperative survival. But we want to inform them the duration of smoking cessation that can provide patients with NSCLC and smoking history similar postoperative prognosis as that of non-smokers. That would potentially encourage current smokers to quit smoking.

To emphasize these points, we have added the following sentences in the revised manuscript.

Changes in the text:
Additionally, there is an increase in the number of smokers who quit smoking owing to the worldwide educational efforts regarding the importance of smoking cessation as a means of preventing lung as well as other cancers (8, 13). Unfortunately, some ex-smokers may still be diagnosed with NSCLC. In order to encourage current smokers to quit smoking, we should be able to provide information regarding the duration of smoking cessation prior to the diagnosis of stage I NSCLC that may result in outcomes and prognosis in smokers similar to that in non-smokers. However, this information is currently unavailable. (page 5, lines 58-63)

Relationship between pathological factors and smoking status
The statistical relationship between clinical and pathological factors among patients with adenocarcinoma and non-adenocarcinoma are presented in Table 3. While not only invasive tumor size but also tobacco habit was significantly correlated with pathological factors among adenocarcinoma patients, there was no such relationship in patients with non-adenocarcinomas. These results indicate that smoking may contribute to the progression of adenocarcinoma. (pages 9-10, lines 125-130)

Ideally, all smokers should quit smoking for the prevention of NSCLC. Although there is a reduction in the numbers of smokers worldwide, several still love smoking (21). There is inadequate information regarding the duration of smoking cessation prior to the diagnosis of stage I NSCLC that may result in outcomes and prognosis in smokers similar to that in non-smokers. Such information, if available, may be useful in encouraging smokers to quit. (page 13, lines 193-196)

12. All page numbers are 14; please correct

Reply 12: Thank you for this comment. We have corrected the page numbers in the revised manuscript.