Peer review file

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Reviewer A

Comment 1: Why was stage not included in the multivariate analysis (Table 3)? Clearly stage is related to recurrence and survival. And according to table 2, CAR, PLR, GPS, and mGPS are all higher in patients with stage II/III. Are the results noted in the multivariate analysis simply due to patients with higher inflammatory markers having higher cancer stage? Are higher inflammatory markers/scores simply a surrogate marker of higher stage? I would strongly suggest including stage in your multivariate analysis.

Reply 1: As suggested, we have now performed an additional multivariate analysis while adding the pathological stage. The CAR (HR: 1.987, 95% CI: 1.202- 3.284, p=0.007) was found to be an independent prognostic factor for the RFS. This result indicated that inflammation-based scores, especially the CAR, represent a reliable supportive prognostic parameter for identifying patients at risk of early recurrence within the same disease stage.

Changes in the text: We have modified Table 3. We also revised the following text:

> Multivariate analyses showed that an elevated CAR (hazard ratio [HR], 1.987; 95% confidence interval [CI], 1.202-3.284) independently predicted the recurrence-free survival. (see Page 4, line 49-51)

> the CAR (p=0.007) was found to be an independent prognostic factor (Table 3). (see Page 15, line 188-189)

Furthermore, the CAR was an independent predictor of the RFS, (see Page 16, line 204-205)

In conclusion, elevated CAR value was significantly associated with a poor RFS, (see Page 21, line 289)

Comment 2: The curves in figure 3, I believe are not adjusted for stage. It is difficult to ascertain whether there is a statistically significant difference for CAR and PLR, after stratifying by stage in figure 4. There needs to be further clarification of figures 3 and 4 regarding whether the differences seen in the curves is a significant difference or not.

Reply 2: We conducted additional hazard curve analyses and an ROC curve analysis by excluding patients with stage IV and those with recurrence within three months after surgery (n=387, see Fig. 1). These analyses revealed a difference in the timing of recurrence between patients with high and low SII values in stage I (Fig. 4, 5). No difference was found in RFS and the timing of recurrence between patients with elevated PLR value and those with low value.

Changes in the text: As suggested, we have replaced figures 3 and 4 with new ones (Fig. 4, 5), and modified our manuscript as follows:

Even in stage I disease, patients with elevated CAR and SII values showed an earlier peak of recurrence, which was about 12 to 16 months earlier than those with low values. (see Page 4, line 51-53)

The optimal thresholds for the CAR, NLR, PLR, SII, and ALI based on ROC curve analysis were set at 0.014, 2.90, 104, 715, and 37, respectively. (see Page 14, line 171-172)

The resulting hazard curves in patients with elevated CAR and SII values showed an initial sharp and high peak within one year after surgery, indicative of early recurrence**. In contrast, t**he peak of patients with low values had a relatively wide, gentle slope (Fig. 4). (see Page 14, line 180-183)

In patients with stage I, the hazard rate curve showed an initial high peak around 12 months after surgery for patients with elevated CAR and SII values (Fig. 5A, C). In contrast, patients with low values had a relatively wide gentle slope, which peaked about 12 to 16 months later than the peak in those with elevated CAR and SII values. (see Page 15, line 193-196)

The SII is calculated using the following formula: platelets × neutrophils / lymphocytes. An elevated SII usually suggests a higher level of inflammation and a lower level of immunity in patients (18). (see Page 20, line 274-277)

Comment 3: There is no mention in the results section about any differences in the hazard curves in stage II/III disease. Did the authors feel that the qualitative difference seen in Fig 4 b and d were not significant? This needs to be clarified.

Reply 3: We thank the reviewer for this comment.

Changes in the text: To clarify this point, we have modified the Results as follows:

The hazard curves of CAR and SII according to the pathological stage were shown to be similar (highest peak around one year after surgery) for both patients with elevated values and those with low values in patients with stage IIA or higher (Fig. 5B, D) (see Page 15, line 190-193).

Comment 4: In the discussion, page 15, line 206, authors state CAR and PLR were independent predictors of RFS. See comment #1 above. Staging information needs to be included in the model before this can be stated as stage is related to both the predictor (see table 2) and is known to be related to the outcomes, recurrence.

Reply 4: An additional multivariate analysis including the pathological stage showed that the CAR independently predicted the recurrence-free survival (see Reply 1).

Comment 5: My biggest concern is that several studies have shown elevated inflammatory markers to be associated with worse survival. The authors state the novel finding in this study is the analysis of the temporal relationship between the inflammatory scores and recurrence. However, as stated above, this is not clearly described and I am not sure if this finding is significant or not. This needs to be clarified to make the results truly novel.

Reply 5: We agree that the overall survival remains one of the best measures for evaluating the prognosis. We performed univariate and multivariate analyses of the overall survival, and the CAR was also found to be an independent predictor of the overall survival (hazard ratio [HR], 2.352; 95% confidence interval [CI], 1.394-3.966; p=0.001). However, our present study aimed to identify patients at high risk of developing early recurrence and focused on the RFS and when recurrence was most likely to occur. Thus, we wish to retain the original text.

Comment 6: Authors should explain why 5 patients with stage IV disease underwent resection. I would consider excluding these patients from the analysis. Their survival is largely determined by metastatic spread of cancer.

Reply 6: As suggested, we performed re-analyses by excluding patients with stage IV

and those with recurrence within 3 months (n=387). The results after these cases had been excluded showed that patients with high CAR, SII, and mGPS values had a poorer RFS than those with low values. Moreover, patients with high CAR and SII values had an earlier peak of recurrence than those with low values in stage I.

Changes in the text: We have now replaced all tables and figures with new ones.

Minor comments:

Comment 1: Page 10, line 103 January is misspelled.

Reply 1: We've corrected the misspelled word.

Changes in the text: "January" (see Page 10, line 103)

Comment2: Page 12, line 152, presented is misspelled.

Reply 2: We've corrected the misspelled word.

Changes in the text: "presented" (see Page 12, line 148)

Comment 3: Please clarify if recurrence is local recurrence, distant recurrence, or both.

Reply 3: We thank the reviewer for this comment.

Changes in the text: As suggested, we have modified the following text:

> One hundred and five (27.1%) of the 387 patients experienced recurrence (local recurrence in 43 patients and either distant metastasis alone or both local recurrence and distant metastasis in 62 patients). (see Page 15, line 175-177)

Reviewer B

Comment 1: The number of covariates highlighted in the CONCLUDION is different between ABSTRACT (4) and RESULTS (2). Please match the two.

Reply 1: We appreciate the reviewer's comment on this point.

Changes in the text: As indicated, we have modified the Conclusions section as follows:

> **Conclusions:** Even after complete resection of stage I NSCLC, patients with elevated CAR and SII values retain a high risk of early recurrence. (see Page 5, line 54-55)

> In conclusion, an elevated CAR value was significantly associated with a poor RFS, and patients with elevated CAR and SII values retain a high risk of early recurrence even after complete resection of stage I NSCLC. (see Page 21, line 289-291)

Comment 2: On page 13 (line 166), it is advisable to display details instead of descriptions such as 'after adjustments for known prognostic factors'. In particular, please describe the way how you adjusted the stage and age of patients.

Reply 2: We agree with the reviewer.

Changes in the text: As suggested, we have revised the text as follows:

> A multivariate Cox regression analysis adjusting for the age, sex, smoking status, BMI, surgery type, surgical procedure, and pathological stage was performed to evaluate the relationship between inflammation-based scores and the RFS. (see Page 13, line 158-161)

Comment 3: On page 15 (line 201), you may need to revise the description, such as 'the risk curve of the initial NSCLC', as this study includes patients with stage IV.

Reply 3: We thank the reviewer for this comment.

Changes in the text: As indicated, we have excluded patients with stage IV, and modified our manuscript from "in early-stage NSCLC patients" to "in patients with completely resected NSCLC". (see Page 16, line 200-201)

Comment 4: On page 30 (Table 1), patients with advanced stages, which are not good for therapeutic resection, were included in this study. So, the facts may have affected the findings of this study.

Reply 4: As suggested, we performed re-analyses by excluding patients with stage IV disease and those with very short time to recurrence (within 3 months after surgery). The results after these cases had been excluded showed that patients with high CAR and SII values had a poorer RFS and an earlier peak of recurrence than those with low values.

Changes in the text: We have now replaced all tables and figures with new ones. We also revised the following text:

A total of 387 patients with NSCLC (see Page 4, line 41)

Median follow-up was 39.2 months (see Page 4, line 47)

Finally, 387 patients (233 men, 154 women) (see Page 10, line 108)

The characteristics of the patients are summarized in Table 1. The median follow-

up period for the 387 patients was 39.2 (range, 3 to 117) months, and the median age was 71 (range, 19 to 86) years old. (see Page 14, line 168-170)

Comment 5: On page 34 (Table 3), it is recommended to describe all the covariates included in the univariate analysis in the METHODS section.

Reply 5: We appreciate the reviewer's comment on this point.

Changes in the text: Accordingly, we have modified Table 3.

Comment 6: The results of this manuscript need to be verified. If you have tried validation, please describe your method and results in the RESULTS section.

Reply 6: We thank the reviewer for this comment.

Changes in the text: As indicated, we have added the following text to the end of the Methods section:

A professional statistician reviewed and verified the statistical analyses. (see Page. 13, line 163-164)

Reviewer C

Comment 1: This paper needs statistical review to ensure proper smoothed hazard estimate analyses.

Reply 1: We appreciate the reviewer's comment on this point.

Changes in the text: As indicated, we have added the following text to the end of the Methods section:

> A professional statistician reviewed and verified the statistical analyses. (see Page. 13, line 163-164)

Comment 2: Patients with wedges should be excluded, most surgeons do not consider a wedge a proper cancer operation.

Reply 2: An additional univariate analysis showed that patients who underwent sublobar resection (wedge resection or segmentectomy) had a better RFS than those who received lobectomy or pneumonectomy, although no significant difference was observed (p=0.054). In the present study, patients with pure ground-glass opacity (GGO) or some mixed GGO tumors on HRCT were allowed to undergo sublobar resection. These tumors are well known to likely be associated with the lepidic-predominant subtype and classified as low-grade tumors. We consider this to be why patients who underwent sublobar resection had a better RFS than those who underwent lobectomy. Thus, we wish to retain patients who underwent sublobar resection in the study cohort.

Comment 3: The stage IV patients need to be excluded if primary endpoint is recurrence.

Reply 3: As suggested, we performed re-analyses by excluding patients with stage IV

disease.

Changes in the text: Accordingly, we have replaced all tables and figures with new ones.

Comment 4: Information on adequacy of nodal dissection is required to inform the reader that patients were properly staged intraoperatively (nodal count and stations evaluated).

Reply 4: We agree that this point requires clarification. Patients who underwent anatomical resection (i.e. segmentectomy/lobectomy/pneumonectomy) received lymph node dissection or sampling. Unfortunately, information about the pathological findings, such as the nodal count and stations, was not sufficiently available, as this information was not always obtained at the time of surgery.

Comment 5: Were all R0 resections?

Reply 5: Yes; we excluded patients who underwent R1/R2 resection.

Comment 6: Do the inflammatory markers prognosticate OS or disease-specific survival?

Reply 6: We performed univariate and multivariate analyses of the overall survival, and the CAR was also found to be an independent predictor of the overall survival (hazard ratio [HR], 2.352; 95% confidence interval [CI], 1.394-3.966; p=0.001).

Comment 7: Patients with very short time to recurrence must be excluded. I suspect that anyone that recurs in <3 months likely had significant, unidentified, unresected disease burden left at the time of surgery.

Reply 7: As suggested, we performed re-analyses by excluding patients with stage IV disease and those with recurrence within three months after surgery. These analyses revealed that patients with elevated CAR and SII values had a poorer RFS and an earlier peak of recurrence than those with low values. On the other hand, no difference was found in RFS and the timing of recurrence between patients with elevated PLR value and those with low value.

Changes in the text: We have added "those with recurrence within 3 months after surgery," to the Methods section (see Page 10, line 104-105) and replaced all tables and figures with new ones.

Comment 8: Need data on adjuvant therapy is necessary to draw any conclusions here.

Reply 8: One hundred and seven patients (27.6%) received adjuvant chemotherapy. The indication for adjuvant chemotherapy was decided according to the guideline recommended at the time, irrespective of inflammation-based scores. In accordance with the reviewer's comment, we divided cases according to the presence of adjuvant chemotherapy and inflammation-based scores. Fisher's exact test showed no statistically significant differences between patients with and without adjuvant chemotherapy by inflammatory scores (CAR: p=0.908, NLR: p=1.000, PLR: p=0.893, SII: p=0.116, ALI: p=0.423, GPS: p=0.614, mGPS: p=0.679). We also performed a univariate analysis stratified by the pathological stage (stage I/stage ≥IIA). No significant difference was noted in the RFS between adjuvant chemotherapy and observation in either group (p=0.088/0.448, respectively).

Changes in the text: We have now added information about adjuvant chemotherapy to the patient characteristics (Table 1).

Comment 9: Were all biomarker values drawn preoperatively? Within what time frame

prior to surgery?

Reply 9: Yes. In brief, blood data were collected from routine preoperative blood tests within one month prior to surgery.

Comment 10: Follow up protocol is unclear? Are CXR or CT or both obtained?

Reply 10: We agree that this point requires clarification. Basically, either chest X-ray or computed tomography was performed at each follow-up visit.
Changes in the text: To clarify, we have revised our manuscript as follows:

> Follow-up evaluations included physical examination, chest radiography, and CT scanning of the chest and abdomen. In general, CT was performed every six months in the first two years after surgery and annually thereafter. (see Page 12, line 136-138)

Comment 11: It would be interesting to see serial inflammatory biomarker values over time. Do they begin to rise again prior to clinically-identified recurrence?

Reply 11: The temporal variation in the patterns of inflammation-based scores throughout the follow-up period would be interesting to know. However, inflammation-based scores were obtained only preoperatively in this study, so we would like to consider exploring this point in a future work.

Comment 12: It appears that some late stage patients have a LATE hazard estimate peak after the initial peak. What do we do with this information? Intensify surveillance early then again late?

Reply 12: Our present findings suggest that appropriate CT-based imaging studies should be performed at the time points showing peaks in the hazard curves during the follow-up period. However, at present, it remains unclear whether the early detection of recurrence contributes to improved outcomes. Based on currently recommended guidelines, follow-up strategies should be designed in a case-by-case basis, considering the cost-benefits and patient satisfaction.

Reviewer D

Minor remarks

Comment 1: Page 12, 152 spell check.

Reply 1: We've corrected the misspelled word.

Changes in the text: "presented" (see Page 12, line 148)

Major remarks

Comment 1: Please provide a study flowchart illustrating the inclusion of patients in the study group.

Reply 1: We agree with the reviewer.

Changes in the text: As suggested, we have added Fig. 1.

Comment 2: What were the methods of obtaining follow up?

Reply 2: The postoperative follow-up schedule consisted of hospital visits to collect the patient's history, performing a physical examination, and imaging studies every three to six months through the fifth year and annually thereafter.

Comment 3: How many patients were lost to follow up?

Reply 3: We defined loss to follow-up as patients with less than 36 months of scheduled hospital visit without an event (recurrence or death due to any cause). The number of patients lost to follow-up was 26 (6.7%).

Comment 4: The multivariable analysis of survival without the inclusion of the most significant prognosticators as pTNM is defective.

Reply 4: As suggested, we have now performed an additional multivariate analysis while adding the pathological stage. The CAR (HR: 1.987, 95% CI: 1.202- 3.284, p=0.007) was found to be an independent prognostic factor for the RFS.

Changes in the text: We have modified Table 3. We also revised the following text:

> Multivariate analyses showed that an elevated CAR (hazard ratio [HR], 1.987; 95% confidence interval [CI], 1.202-3.284) independently predicted the recurrence-free survival. (see Page 4, line 49-51)
>
> the CAR (p=0.007) was found to be an independent prognostic factor (Table 3). (see Page 15, line 188-189)
>
> Furthermore, the CAR was an independent predictor of the RFS, (see Page 16, line 204-205)
>
> In conclusion, elevated CAR value was significantly associated with a poor RFS, (see Page 21, line 289)

Comment 5: The patients with other diseases modifying the inflammatory response (rheumatoid arthritis, neoplasms, hematologic disorders, associated TB, empyema, etc., pneumonia) were not excluded. Please comment on that.

Reply 5: We agree that this point requires clarification. However, patients with hematologic disorder who require no treatment, a history of TB, and mild rheumatoid arthritis were not excluded from this study.

Changes in the text: To clarify this point, we have modified our manuscript as follows:

those with acute infection (e.g. empyema, pneumonia), those with active double cancer, (see Page 10, line 105-106)

Comment 6: Smoking status is a significant factor influencing inflammation. The patients are not characterized in this field. Please elaborate on that.

Reply 6: We agree that the smoking status is important information to consider when interpreting the results of the present study. There were statistically significant differences between never smokers and smokers in some inflammation-based scores (CAR: p< 0.001, PLR: p=0.022, GPS: p=0.37, mGPS: p< 0.001, Fisher's exact test). However, smoking status was not a significant independent prognostic factor for RFS in multivariate analysis.

Changes in the text: We have now added the smoking status to the clinicopathological characteristics (Table 1) and modified Table 3.

Comment 7: It is difficult to conclude about the true meaning of the prognosticators in different stages of NSCLC. Please have in mind that some of the lab factors correlate with the NSCLC stage. I insist on either multivariable analysis including pTNM, surgery type, smoking status, comorbidities, or performing another ROC curves and survival analysis on a very homogenous population of patients with stage I without current smoking, significant comorbidities, and treated with VATS lobectomy.

Reply 7: As suggested, we performed an additional multivariate analysis including the pathological stage, surgery type, surgical procedure, and smoking status. The CAR was found to be an independent prognostic factor for the RFS. (See Reply 4).