Peer Review File
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Reviewer A

In their paper the authors have retrospectively analyzed patients who underwent upfront surgery for stage I-III NSCLC between 1998-2021.

Comment 1:
• My first consideration is about the wide time frame: big changes in both surgical approach and chemotherapy regimen have taken place. A comment should be added on that.

Reply 1:
Thank you to reviewer A for taking the time to provide insightful comments relating to our work. We absolutely agree that this time frame is rather wide. We chose such a wide time frame in order to capture enough patients to achieve a sample size capable of deciphering changes in the circulomic milieu and their potential association with disease biology. Given this issue, the regression model was corrected for surgical era, divided into quartiles, to ensure that changes in surgical approach may be reflected in the final analysis. We apologize that this wasn't immediately clear in the prior version of the manuscript. We've explicitly clarified this issue in the revised manuscript. Regarding chemotherapy, we have clarified that none of the patients in this cohort received neoadjuvant chemotherapy as we acknowledge that this would confound the analysis.

Changes in the text:
See lines 240-241.

Comment 2
• Line 130 and following: "Clinical and pathological stages were defined using the 8th edition of the American Joint Commission on Cancer". Does it mean that the 1998 specimen were restaged according to the new TNM edition? The pathologists do that? As the upstaging is the main important finding of this analysis, a precise definition on this aspect is mandatory.

Reply 2:
Thank you for bringing this up to our attention. This is an incredibly important question, and clarification is needed in our text. We used available data from the electronic medical record in order to translate stage of disease according to the 8th edition of the AJCC, so that consistent staging definitions could be used in the model. However, given the changes in staging over time, with some particularly granular pieces of data unavailable, only "whole" stages could be considered, such as Stage I, II, and III, rather than stage Ia, Ib, etc.. Regardless, given the sample size, we believe that evaluating the

stage at this level is adequate.
Changes in the text:
See line 126.

Comment 3:
• Line 158 and following: Did the patient undergo PET-CT scan? The definition of preoperative assessment and the practice to MDT case discussion should be included in the Method section as this can impact the precise clinical staging.

Reply 3:
We appreciate this excellent point with regard to the quality of preoperative assessment. Pathologic stage was provided by evaluation of surgical specimens. However, the pre-operative therapy strategy was based on clinical staging, expert thoracic surgeon opinion, and multidisciplinary discussions as appropriate. It is routine in our practice to perform PET-CT, brain MRI, and histologic mediastinal nodal staging for clinical staging. We have addended our methods section in our revised manuscript to further describe the diagnostic and therapeutic pathways used in the perioperative period.

Changes in text:
See lines 125-128.

Comment 4:
Data on 4141 patients were analyzed with particular attention to the circulomic variables (platelet and lymphocyte count from the last blood draw prior to resection and platelet-to-lymphocyte ratio -PLR-) Patients with elevated PLR were found to have reduced risk of upstaging (OR= 0.757, 46 CI: 0.650-0.882). In my opinion it is a really interesting finding.

Reply 4
We greatly appreciate this comment. We agree, this finding is especially relevant given the increased work surrounding the role of platelets in oncogenesis and response to therapy.

Reviewer B

The paper was well written and easy to follow but may still benefit from considering the following comments and questions.

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

Reply 1:
We thank this Reviewer for the suggested addition of a consort diagram in our manuscript. We have provided a study flowchart in the revised manuscript

Changes in text:
Please see figure 1, which represents the included cohort.

Comment 2:
The patients with other diseases modifying the PLR (hematologic disorders, empyema, pneumonia, etc.,) were not excluded. Please comment on that.

Reply 2:
Thank you for this thoughtful comment. We agree that other coexisting diseases may impact the PLR, such as hematologic (platelet) or infectious (lymphocyte) disorders. We've captured the PLR at the time prior to resection, and, as such, any post-operative hematologic (bleeding) or infectious (SSI) disorders would not have impacted this metric. To this reviewer's point, our data did not capture whether patients presented with pre-existing disorders, that may have impacted the PLR. As such, we've addended our discussion, specifically, our limitations, to reflect the above discussion, which we believe adds greatly to the revised manuscript. .

Changes in text:
See lines 245-247.

Comment 3:
The staging protocol lacks some granularity in its description. Were these patients exclusively staged by Thin-section CT and/ or PET? What were the indications for EBUS/ mediastinoscopy?

Reply 3:
Our standard workup includes thin-cut CT scan, PET/CT, brain MRI, and invasive mediastinal staging with EBUS or mediastinoscopy. We've addended the methods and discussion sections of our revised manuscript to reflect clarifying granularity in the staging protocol employed at our institution.

Changes in text:
See lines 125-128.

Comment 4:
Could pre-operative biopsy with histological diagnosis and classification affect the PLR score? Please comment on that.

Reply 4:
Yes, it is theoretically possible that biopsy may impact PLR. However, biopsies were employed for most patients in the modern era and across groups equally. With regard to histological classification, we've performed a new analysis to address the reviewer's comment. In comparing the PLR across histopathologies, we found that patients with

squamous cell carcinoma had higher PLRs compared to patients with adenocarcinoma or other histopathologies ($p<0.001$). We controlled for histology in assessing the impact of circulomic characteristics on pathologic upstaging, and thus, this is reflected in the multivariable model, with squamous histology being "protected." Additionally, we've also performed a mediation analysis that showed that histopathology's effect on upstage was minimally (5.37%) and insignificantly ($p=0.113$) mediated by PLR. However, this was not part of our objectives in this study and so we are reluctant to add these analyses in the manuscript, which may take away from the main message, unless the reviewer and editor feel that this would improve our text.

Changes in text:
None

Comment 5:
Were all R0 resections?

Reply 5:
We included both negative and positive resections in this analysis as we do not think that this would have an impact on the rate of upstaging as this is an event that technically occurs following the occurrence of upstaging. The rate of R+ margins in patients who were not upstaged was 2.2% compared to 3.3% in those who were upstaged ($p=0.054$). We've addended the manuscript to describe this thought process with more clarity.

Changes in text:
Kindly see lines 115-116

Comment 6:
Information on adequacy of nodal dissection is required to inform the reader that patients were properly staged intraoperatively (nodal count and stations evaluated).

Reply 6:
As a result of the reviewer's comment, we went back and evaluated both number of stations evaluated and number of nodes evaluated. In both cohorts, the median number of stations evaluated was 4. The median lymph nodes checked in patients who were not upstaged was 10 (interquartile range [IQR]: 4-16), compared to 13 in patients who were ultimately upstaged (IQR: 7-19), likely correlating with clinical gestalt regarding disease aggressivity. This comment has led to a new analysis that we will include in our revised results and in our addended table. This has tremendously strengthened the manuscript and we thank the reviewer for their thoughtful comment.

Changes in text:
Kindly see changes on lines 129-130, 172-175, and in our revised table 1.

Comment 7:

How does PLR correlate with pathological findings (histopathology, tumor size, pleural invasion, LVI, NVI, etc.)?

Reply 7:
Thank you for this excellent question. The current research question encompassed the effect of circulome characteristics on rates of upstaging. This is merely a reflection of disease pathology which potentially would include a correlation with histopathology, tumor size, LVI, NVI, ect. However, despite controlling for the characteristics, the current manuscript highlights that PLR is independently associated with rate of upstaging. Future work will evaluate the effect of histopathology, tumor size, LVI, NVI, and even receipt of chemotherapy (this cohort was treatment naïve), on circulomic characteristics. We've previously shown that surgical approach does not impact circulomic characteristics (PMID: 35038676), however, a lot of work is needed in this context, and we thank the reviewer for asking this thoughtful question. We've addended the discussion section of our revised manuscript to reflect this thought process.

Changes in text:
Please see changes on lines 244-245