Peer Review File

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Reviewer A

This manuscript aimed to investigate whether serum calcium level predicts disease-free survival in 508 patients who diagnosed with pathologic stage I-III after surgery for rectal cancer.
This manuscript has the advantage of obtaining and presenting significant results using various statistical analysis methods.
However, the following ambiguous parts or corrections exist in the manuscript.
1. Among the many time points, is there a reason for collecting blood samples before surgery to measure serum calcium and PLR? For example, if the author wants to analyze systemic inflammation before definitive treatment, isn't it correct to base blood tests taken before neoadjuvant treatment? Conversely, if you want after definitive treatment, isn't it correct to use blood tests taken at the time point after adjuvant treatment?
Reply: We appreciate your critical considerations. In this cohort, it's a routine pathway to do blood tests, including serum calcium, platelet and lymphocyte, in patients undergoing rectal cancer resection before and immediately after surgery during the period of weeks according to the institutional guidelines for perioperative care. In this study, we consider the perioperative care as a critical period for anti-cancer treatment that involves in host-immune and inflammatory process for control and development of potential circulating cancer cells, distant minimal metastasis and minimally residual cancer lesions. Therefore, we can apply these preoperative data to perform the analysis in our study. We have mentioned these information in the Method section (see page 4 and 5).

2. Describe the abbreviations such as AIC and LR in abstract.
Reply: We appreciate your careful review. We should have provided full names of these abbreviations. We have added the abbreviations in the revised abstract (see page 2 line8).

3. Describe the follow-up duration.
Reply: We appreciate your careful review. We have mentioned the follow-up duration in the Method section (see page 5 line 16-22):
Patients were followed up every three months for the first two years and every six months thereafter. Each visit consisted of pertinent medical history, physical examination, including rectal examination, and measurement of serum carcinoembryonic antigen (CEA) levels. Colonoscopy and radiological examinations consisting of chest radiography, abdominopelvic CT and ultrasonography were scheduled every six months for the first three years and annually thereafter. Cancer recurrence was detected by CEA > 5 ng/mL and/or a sequential computerized tomography scan with evidence of the disease followed by histopathological confirmation.

Reviewer B

I found the paper to be overall well written and much of it to be well described. I recommend that a minor revision is warranted. I explain my concerns below and ask that the authors specifically address each of my comments in their response.

Minor comments:

1. Systemic inflammation and immune response play crucial roles in tumor growth; glasgow prognostic score(GPS), prognostic nutritional index(PNI), neutrophil-to-lymphocyte ratio(NLR), sarcopenia, etc. are widely known systemic inflammatory scoring system. Is there any special reason for selected PLR as an indicator for systemic inflammation marker in this manuscript?

Reply: We appreciate your critical considerations. We do have a full consideration on selection of systemic inflammation markers at the initial design stage of this study.

In this cohort, according to the institutional guidelines for perioperative care, all patients received blood tests, including serum calcium, platelet and lymphocyte, before and after surgery. However, the serum C-reactive protein (CRP) was not included as a routine test in the perioperative care protocol. Thus, the information of CRP was absent in the majority of the patients in this cohort, and we could not investigate glasgow prognostic score (GPS) and prognostic inflammatory and nutritional index (PINI) that aggregate CRP as combine models. In addition, the is not a well-studied systematic inflammation index in the previous colorectal cancer literature.

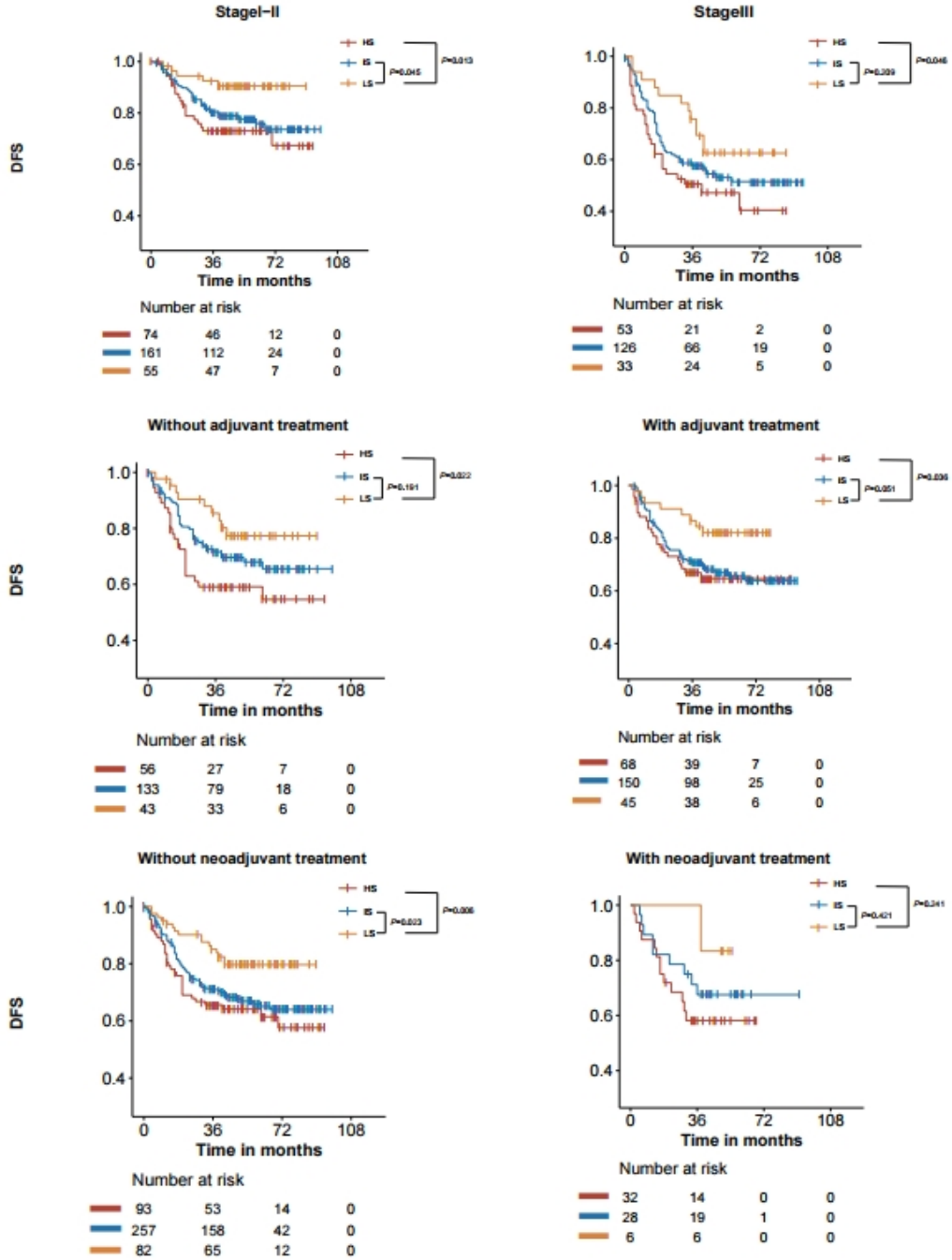
For neutrophil-to-lymphocyte ratio (NLR) and prognostic nutritional index (PNI) you kindly mentioned, we did have initially included NLR, PNI and PLR, respectively, in the model generation. However, the results showed that NLR is not well performed in this study, and CaPLR performed better than CaNLR and CaPNI (a model combined serum calcium and NLR or PNI) in our preliminary test. This is not unexpected when the results of NLR was referred to some previous publications demonstrating that the prognostic value of NLR[1-3] or PNI[4] is inconsistent in mulitple published cohorts. In addition, it has been shown that PLR performed better in predicting both prognosis and long-term outcomes[5]. Recent studies have reported the association between calcium and platelet and its potential biological mechanism [6, 7], which may support our predicting panel that combines calcium with platelet-based PLR.

2. The topic addressed is interesting and deserves a constructive discussion. Was there a difference in postoperative prognosis due to CaPLR scoring by stage? Also, was there a difference in prognosis with or without neoadjuvant/adjuvant chemotherapy? Although it is shown as 'the survival outcomes after surgery vary in different patients, which makes it essential to stratify patients by different risk of recurrence and death to avoid overtreatment or insufficient therapy (page2, line4-)', isn't it better to show your therapeutic indication for rectal cancer?

Reply: We appreciate your good suggestion that improves the robustness of our generated CaPLR model. To answer this question, we did a series of subset analysis based on stage, neoadjuvant chemotherapy and adjuvant chemotherapy. We have provided the result in the resubmitted files. According to the results, in each subset with different TNM stages, adjuvant treatment, or

neoadjuvant treatment, each CaPLR group had significantly different disease-free survival outcomes, especially for patients in HS group (vs. LS group). For patients without neoadjuvant treatment, the LS group still have a significantly better DFS; while for patients with neoadjuvant treatment, there is no significance between different CaPLR group, which may attributed to the limited sample size.

Patients receiving adjuvant treatment always have more well-documented clinicopathological features that are associated with high risk of recurrence according to the standard NCCN guideline-based treatment protocols, and a comparison of survival outcomes between patients receiving and not receiving adjuvant treatment may be confounded or impacted by high-recurrence-risk clinicopathological features and cannot accurately reflect the effectiveness of chemo(radio)therapy and biomarker stratification in a retrospective and observational study. Thus, it would be better to design a prospective trial to investigate CaPLR as a biomarker for chemo(radio)therapy. We also considered a validation in a lager cohort in the future studies.



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