January 2, 2023

Dear editor:

Thank you for your encouraging letter concerning our manuscript entitled “Risk Factors Predict Microscopic Extranodal Tumor Deposits in Advanced Stage III Colon Cancer Patients” by Yi-Han Jhuang et al.
We are extremely grateful to you and the reviewers for the constructive critique of our manuscript. We have responded to each of the comments of the referees on separate sheets and deeply appreciated your suggestions that have led to a significant improvement in this article. All the changes are labeled in track changes.
We look forward to your prompt reply.

Yours sincerely,

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Answer to Editor's and Reviewer's comments

Thank you for your positive comments on this manuscript. The responses to the raised questions are as below.

Comments from Reviewer #1 (Number ID: 06269450):

1. The results in the Abstract section are incorrect, please check and modify.

-Response: Thank you for your suggestion. We had revised our results in the abstract section. (Page 3, line 44-45, 50-51, 54-55)

2. The manuscript only lists the exclusion criteria and does not write the specific inclusion criteria of patients.

-Response: Thank you for you r suggestion. We revised our methods section. The patients included in our study had completed the colonoscopy with tumor biopsy and pathology reported malignancy. Besides, abdomen computed tomography and whole-body positron emission tomography proved no distant metastases. All patients followed at out-patient department regularly. (Page 6, line 96-101)

3. The results of the study only analyzed the relationship between the overall survival rates and LVI, the relationship between disease-free survival rates and LVI. Conclusion How did the conclusions arrive at the relationship between LVI and TDs and how TDs combined with LVI can predict poor patient outcomes, please conduct more detailed and accurate analysis.

-Response: Thank you for your suggestion. The overall survival rates and disease-free survival rates in our study were lower in the patient with LVI, which was compatible with the previous studies. Furthermore, we investigated the impact of LVI on the N1c and non-N1c subgroups using the multivariate cox regression analysis and Kaplan–Meier method, which found the patient with both LVI and TDs had lower overall survival (P=0.01). (Page 11, lines 172-174, 176-178)

Comments from Reviewer #2 (Number ID: 06290122):

The authors conducted a retrospective study to investigate the risk factors in stage III colon cancer with extranodal TDs. The research is derived from AJCC TNM staging on TD in colon cancer. The paper is well-written, and the research is interesting.

1. However, in the N1c with LVI subgroup, the sample size is just 3 cases, and the sum is too small, which may result in serious bias for the conclusion.

-Response: Thank you for your suggestion. The prevalence of N1c in colon cancer could low as 1.59% in the previous study and our study also revealed the same small

sample size. However, the Figure 2c and Figure 3c all showed apparent “separated curves”. The Figure 2c showed that N1c patient with LVI had significantly lower overall survival rates than those who without. ($P<0.05$). Although the Figure 3c showed no significant result, but the p value was very close to 0.1, which might be due to our limited case number. Further prospective studies with more patients involved might address the result more promising, and we had addressed the limitation of the study in the discussion part. (Page 15-16, line 257-261)

2. In addition, in your figures, you should present the HE staining of TD and non-TD.
-Response: Thank you for your suggestion. We present the HE staining of TDs and non-TDs as Figure 4a and 4b. The description is also revised in the Methods section. (Page 7, line 118-122). Besides, the pathologic photos were provided by the pathologist, we also added him as the coauthor in our manuscript. (Page 1, lines 4, 16-17)

Comments from Reviewer #3 (Number ID: 06393261):

1. The aim you reported in the abstract is not relevant to the manuscript. I propose “We conducted a retrospective study to investigate risk factors for extra nodal TD in stage III colon cancer” rather than "We conducted a retrospective study to investigate the risk factors in stage III colon cancer with extranodal TDs".
-Response: Thank you for your suggestion. We revised our abstract section as your recommendation. (Page 2-3, line 36-37)

2. What do you mean by “adjusting post-operative chemotherapy”? In your methodology, you only include stage III colon cancer, which itself is an indication for chemotherapy. You mean switch to another protocol, add immunotherapy? I suggest you rephrase this sentence.
-Response: Thank you for your suggestion. We revised our introduction section. We correlated the role of extranodal TDs with the prognosis of advanced stage III CRC patients, hoping to provide further information for adjusting the chemotherapy regimens with possible target therapy or immunotherapy. (Page 5-6, line 87-90)

3. Study design and population: You should specify that the diagnosis is based primarily on the pathological report. CT scan and PET scan are mainly done to eliminate a stage IV tumor, which does not meet the inclusion criteria.
-Response: Thank you for your suggestion. We revised our methods section. The patients included in our study had completed the colonoscopy with tumor biopsy and pathology reported malignancy. Besides, abdomen computed tomography and

whole-body positron emission tomography proved no distant metastases. All patients followed at out-patient department regularly. (Page 6, line 96-101)

4. You reported that "some studies have even stated that TDs and tumor budding are the only histological variables that independently predict tumor recurrence in stage III colon cancer". However, Nagayoshi & al (reference 8) found that "Tumor deposit was an independent prognostic factor in N0 and N1 colorectal cancer, whereas N2 cancer had poor survival outcome regardless of tumor deposit." This reference (N° 8) should be revised. Otherwise, it may confirm the results you found.
-Response: Thank you for your suggestion. We revised this section and acknowledged that N2 cancer had poor survival outcome regardless of tumor deposit which might be conflict with our result. Some studies have even stated that TDs and tumor budding are the only histological variables that independently predict tumor recurrence in stage III colon cancer and should be included as part of a routine comprehensive pathological risk assessment. TDs are defined as a discrete focus of tumor within the lymph node drainage area of the primary carcinoma with no identifiable lymph node (Page 12, line 197-202)

5. You found no statistically significant difference between overall free- survival among patients with and without LVI within N1c group ($p=0.097 > 0.05$). "We showed that LVI could predict CRC in patients with the N1c component", this sentence must be expressed differently. LVI cannot predict CRC, since patient were operated with the diagnosis of a colon carcinoma.
-Response: Thank you for your suggestion. We revised our sentence as “We showed that LVI could predict CRC patients with N1c component” in the discussion section. (Page 14, line 225-226)

Reference:

1. Simon HL, Reif de Paula T, Spigel ZA, Keller DS. N1c colon cancer and the use of adjuvant chemotherapy: a current audit of the National Cancer Database. Colorectal Dis. 2021 Mar;23(3):653-663. doi: 10.1111/codi.15406. Epub 2020 Nov 24. PMID: 33064353.