Author's response to reviews

Title: The impact of chemotherapy-associated neutrophil/lymphocyte counts on the efficacy of adjuvant chemotherapy in colorectal cancer

Authors:

Chu-Yuan Hong (hongchuyuan@126.com)
Jing Peng (ykkhds1998@sina.com)
Yi-Sheng Wei (yswei2004@126.com)
He-Peng Peng (penghp@yeah.net)
Hui Yang (yanghui030454@gmail.com)
Chu-Xiong Zhao (zhaochux@126.com)
Guo-Jian Liang (854768420@qq.com)
Guo-Qiang Wang (wqqiyh@163.com)

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Author's response to reviews: see over
Dear Prof. Tonilynn Manibo, Prof. Milo Frattini, and Editors:

Thanks for inviting to revise our manuscript (Manuscript ID: 1926266349704115) entitled “The impact of chemotherapy-associated neutrophil/lymphocyte counts on the efficacy of adjuvant chemotherapy in colorectal cancer” submitted by Hong et al. to BMC Cancer for publication.

Under the editor and reviewers’ comments, we have performed more statistics, revision, modification, and analysis as follows:

(a) We have presented OS data and evaluated the impact of chemotherapy-associated neutrophil/lymphocyte counts on OS.

(b) We have changed “predictive value” to “prognostic value”. Moreover, the title has been changed to “The impact of chemotherapy-associated neutrophil/lymphocyte counts on prognosis of adjuvant chemotherapy in colorectal cancer”.

(c) We have mentioned it is a retrospective study.

(d) We have incorporated the duration of lymphopenia in the multivariate cox regression model.

(e) We have reported doses of 5-FU and oxaliplatin, the baseline neutrophil/lymphocyte counts.

(f) We have revised the tables and figures.

(g) We have asked for the native speakers of English and corrected the spelling and grammatical errors as many as possible. Enclosed please find more detailed revision in our revised manuscript in the form of a clean copy, and a marked copy with all changes by tracks. We also answered reviewers’ comments point by point in our response letter.

We hope we have adequately addressed the reviewers’ concerns. We would like to thank you and the reviewers for all the efforts you have made to improve the quality of the manuscript. We appreciate the opportunity to have the revision to be reconsidered for publication in your journal.

Sincerely,

Yisheng Wei, M.D., Ph.D.

Referee 1

Reviewer’s report:
Minor Essential Revisions

1. The primary end-point of the study was DFS and not tumor recurrence that is mentioned repeatedly throughout the manuscript.

**Response:** Thanks for the reviewer’s comment. DFS/OS has two implications: survival status and survival time. However, tumor recurrence/death has only one implication: recurrence/death status. In Kaplan–Meier method and Cox regression model, we selected DFS, but not tumor recurrence/death as the dependent variable. However, in ROC analysis, only tumor recurrence/death status, but not DFS/OS can be regarded as the dependent variable. Therefore, DFS/OS was the primary end-point in Kaplan–Meier method and Cox regression model, while tumor recurrence/death status was the primary end-point in ROC analysis.

2. The authors should also present OS data, if available, even if the associations with the tested markers are negative.

**Response:** Thanks for the reviewer’s comment. We have presented OS data in our revised manuscript.

3. In the present study, the authors can only evaluate possible prognostic value of the tested markers, not predictive value, as repeatedly stated throughout the manuscript. Please correct.

**Response:** Thanks for the reviewer’s suggestion. We have corrected the mistakes in our revised manuscript.

4. In many of the references the year is missing.

**Response:** Thanks for the reviewer’s comment. We have added the year in the references.

Discretionary Revisions

1. The entire manuscript is replete with spelling and grammatical mistakes. The authors should make a serious effort to correct them. For example:

Page 3. Background is misspelled

Page 5, 1st line should be changed to: ...most likely to be resistant...
Page 7, 3rd and 4th lines should be changed to: "The prognostic value of chemotherapy-associated neutrophil/lymphocyte counts on DFS was evaluated by Receiver Operating..."

A similar correction should be made to the second heading in page 8: "The prognostic value of chemotherapy-associated neutrophil/lymphocyte counts on DFS"

Page 9, 5th line should be changed to: ...which was found by ROC analysis...

**Response:** Thanks for the reviewer’s comments. We have corrected the spelling and grammatical mistakes in our manuscript. However, in our opinion, the grammatical mistake in Page 7, 3rd and 4th lines in our previous manuscript should be changed to: The prognostic value of chemotherapy-associated neutrophil/lymphocyte counts on tumor recurrence, death was analyzed by Receiver Operating Characteristic (ROC) analysis (Page 7, 15th, 16th and 17th lines in the revised manuscript). Similarly, the grammatical mistake in the second heading in page 8 in our previous manuscript should be changed to: the prognostic value of chemotherapy-associated neutrophil/lymphocyte counts on CRC recurrence/death (the second heading in page 8 in the revised manuscript). Just like the response of question 1, tumor recurrence/death status, but not DFS/OS, was the primary end-point in ROC analysis.

**Referee 2**

Major comments

1) Is it a prospective or retrospective study?

**Response:** Thanks for the reviewer’s comment. It is a retrospective study and we have mentioned that in the revised manuscript.

2) It is not reported anything about the duration of lymphopenia. Was that factor significant for the clinical outcome? It should be mentioned.

**Response:** Thanks for the reviewer’s comment. We have incorporated the duration of lymphopenia in the multivariate cox regression model.

3) It is also not reported what was the doses of 5-FU and oxaliplatin which were used for the treatment of the patients. Was there a standard dose common for all patients? What was the treatment provided to the patients with neutropenia? Was there any difference in the clinical outcome between the patients developed
neutropenia only compared to those with neutropenic infection?

Response: Thanks for the reviewer’s comment. We have reported the doses of 5-FU and oxaliplatin in our revised manuscript. The doses of oxaliplatin and 5-FU were reduced by 15% in subsequent cycles due to severe toxicity including grade 3/4 neutropenia. 4 cases suffered from neutropenic infection requiring hospitalization and treatment with intravenous antibiotics. 3 cases with neutropenic infection suffered from tumor recurrence and died. However, the sample size of the cases with neutropenic infection was too small. Therefore, the comparison of the clinical outcome between the patients who developed neutropenia only and those with neutropenic infection may be not reliable. Moreover, such comparison was not the aim of our study. Thus, we have not mentioned the difference in the clinical outcome between the patients who developed neutropenia only compared to those with neutropenic infection.

4) The authors should discuss about the possible mechanisms which may explain their observation. It is not understandable how a transient lymphopenia could be a predictive marker for those patients. How many of those patients had low (# 0.66) absolute number of lymphocytes before treatment? How many of those patients had rectal cancer which means more complex treatment (chemoradiation)?

Response: Thanks for the reviewer’s comment. We have discussed about the possible mechanisms which may explain our observation in the revised manuscript. In fact, we have mentioned that those with adequate baseline bone marrow (absolute baseline neutrophil counts ≥ 2.0×10^9 cells/L, absolute baseline lymphocyte counts ≥1.0×10^9 cells/L, baseline platelet counts ≥100×10^9 cells/L) were included in our study. Therefore, no case had low (# 0.66) absolute number of lymphocytes before treatment. After all, our study focused on the impact of neutrophil/lymphocyte counts during chemotherapy, but not before treatment, on prognosis of CRC. 42(17.3%) cases had chemotherapy-associated lymphopenia<0.66×10^9/L and 90(37.0%) cases had chemotherapy-associated lymphopenia<0.91×10^9/L in our study. Our study included 111 (45.7%) rectal cancer cases who received chemotherapy only after surgery. Our study focused on the impact of chemotherapy-associated neutrophil/lymphocyte counts on prognosis of CRC to guide the individualized medicine in adjuvant chemotherapy...
of CRC. However, radiotherapy may have an impact on the neutrophil/lymphocyte counts. Therefore, those who received concomitant or neoadjuvant radiotherapy were excluded and we have mentioned the exclusion criterion in the manuscript.

5) In the table 2 is shown that CEA, stage of the disease and lymphopenia are independent prognostic variables for DFS. What is the percentage of patients who had all of those negative prognostic factors?

Response: Thanks for the reviewer’s comment. Chemotherapy-associated lymphopenia <0.66×10^9/L, pretreatment CEA ≥10 ng ml^-1, stage III were independent prognostic variables for DFS, and 99 (40.7%) cases had all of those negative prognostic factors. Chemotherapy-associated lymphopenia <0.91×10^9/L, pretreatment CEA ≥10 ng ml^-1, stage III were independent prognostic variables for OS, and 77 (31.7%) cases had all of those negative prognostic factors.

Minor comments

1) English should be improved

Response: Thanks for the reviewer’s comment. We have made a serious effort to correct the spelling and grammatical mistakes.

2) Figures 1 and 2 are not so clear

Response: Thanks for the reviewer’s comment. We have revised Figures 1, 2 and 3.

3) Table 1 should be more comprehensive. It needs improvement, by using bold or italics as headings, in order to become easy readable.

Response: Thanks for the suggestion. We have revised Tables 1, 2, 3, 4 and 5.

Referee 3

Reviewer's report:

This paper describes in a series of colorectal cancer patients the prognostic role of chemotherapy-associated lymphopenia. This is an interesting and important
subject article but the actual study does not meet the high expectations. There are major methodological errors in the way in which the study was performed which make the results unreliable.

Major points:

It is a prospective or a retrospective study? This point must be clarified.

**Response:** Thanks for the reviewer’s comment. It is a retrospective study and we have clarified that in the revised manuscript.

The majority of included patients have stage II colorectal cancer. It is surprising because adjuvant chemotherapy for stage II colon cancer remains still controversial.

**Response:** Thanks for the reviewer’s comment. Our study included stage II patients with at least one of the following high risk factors: T4, tumor perforation, bowel obstruction, poorly differentiated tumor, venous invasion, or less than 10 lymph nodes examined. However, part of stage II patients with no risk factor had the intention to receive chemotherapy. In our hospital, they were informed of the efficacy and risk of chemotherapy and received chemotherapy.

The patient population is not as well characterized as it might be.

**Response:** Thanks for the reviewer’s comment. We have revised the manuscript and table 1 to characterize the patient population better.

The study selection criteria excluded patients who had absolute baseline lymphocyte counts < 1.0×10^9 cells/L. According my point of view, this criteria of exclusion is not justified.

**Response:** Thanks for the reviewer’s comment. Our study focused on chemotherapy-associated neutrophil/lymphocyte counts on prognosis of CRC. The inadequate baseline bone marrow with baseline neutrophil/lymphocyte counts drop may be due to other factors such as inflammation, drugs. Moreover, pretreatment myelosuppression cases with severe baseline neutrophil/lymphocyte counts drop can’t receive chemotherapy until recovery. Therefore, the eligibility criteria included adequate baseline bone marrow (absolute baseline neutrophil counts ≥ 2.0×10^9 cells/L, absolute baseline lymphocyte counts
≥1.0×10^9 cells/L, baseline platelet counts ≥100×10^9 cells/L). The similar eligibility criterion was also included in MOSAIC study (N Engl J Med 2004, 350(23):2343-2351).

- We do not know how many patients with rectal cancer received neoadjuvant (chemo-)radiotherapy. Previous radiotherapy could affect lymphocyte count during adjuvant chemotherapy.

**Response:** Thanks for the reviewer’s comment. There were 111 (45.7%) rectal cancer cases who received chemotherapy only after surgery in our study. No rectal case received neoadjuvant (chemo-)radiotherapy. Our study focused on the impact of chemotherapy-associated neutrophil/lymphocyte counts on prognosis of CRC to guide the individualized medicine in adjuvant chemotherapy of CRC. However, radiotherapy may have an impact on the neutrophil/lymphocyte counts. Therefore, those who received concomitant or neoadjuvant radiotherapy were excluded and we have mentioned the exclusion criterion in the manuscript.

- Baseline lymphocyte counts are not reported in the manuscript.

**Response:** Thanks for the reviewer’s comment. We have reported baseline neutrophil/lymphocyte counts in the revised manuscript.

- We do not know why patients who had to stop adjuvant for severe toxicity were excluded from analysis? According my point of view, exclusion of these patients is not justified.

**Response:** Thanks for the reviewer’s comment. We have included 21 patients who had to stop adjuvant for severe toxicity in the revised manuscript.

Disease-free survival (DFS) as surrogate end-point for overall survival (OS) is considered an accepted end-point for adjuvant trials in colorectal cancer. So, DFS is not more representative in the assessment of adjuvant chemotherapy efficacy. The evaluation of overall survival as secondary end point should be performed.

**Response:** Thanks for the reviewer’s comment. We have presented OS data and evaluated
the prognostic value of chemotherapy-associated neutrophil/lymphocyte counts on OS.

Minor points:
Number of patient with lymphopenia > 660 are not reported in the manuscript.

**Response:** Thanks for the reviewer’s comment. 42(17.3%) cases had chemotherapy-associated lymphopenia<0.66×10^9/L and 90(37.0%) cases had chemotherapy-associated lymphopenia<0.91×10^9/L in our study. That is to say, 201(82.7%) cases had chemotherapy-associated lymphopenia ≥0.66×10^9/L and 153(63.0%) cases had chemotherapy-associated lymphopenia ≥0.91×10^9/L in our study. We have mentioned that in our revised manuscript.

The rate of FOLFOX-associated neutropenia must be discuss (In MOSAIC study, neutropenia grade ¾ rate was observed in 41,1% of patients).

**Response:** Thanks for the reviewer’s comment. We have discussed the rate of FOLFOX-associated neutropenia: The rate of grade 3/4 neutropenia in our study was lower than that in the MOSAIC study (41.1%). In MOSAIC study, FOLFOX was administered to those who have neutrophil counts >1.5×10^9/L, while those with baseline neutrophil counts ≥2.0×10^9 cells/L were included in our study. That may contribute to the difference in the severity of neutropenia

Chemotherapy-associated lymphopenia is reported rarely in clinical trials. This point should be discussed.

**Response:** Thanks for the reviewer’s comment. We have discussed this point in our revised manuscript.