

Format for ANSWERING REVIEWERS



January 20, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7162-review.doc).

Title: Prognostic significance of preoperative fibrinogen for patients with colon cancer

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer code: 00180872

(1) Why 60 is a threshold age?

Answer: First of all, more literatures reported that mean age of colorectal cancer patients age at diagnosis was 60.2 ± 11.7 (y) (Ji Won Park, et al. *Ann Surg Oncol* (2010) 17:2839-2846; WANG Qiong, et al. *Chin J Oncol*, 2005,27(9): 544-546, et al). Secondly, some researchers chose 60y as a threshold for patients with colorectal cancer (such as Son HJ, et al. *Ann Surg Oncol* 2013;20:2908-2913, et al). Finally, In this study, the mean age was (59.47 ± 12.63) ys old.

(2) What about the Gender?

Answer: the number of males and females were 135 cases and 120 cases respectively.

(3) Were all there any pts treated on an emergent basis?

Answer: There were no participator who was treated on an emergent basis through asking their medical history because it maybe influence preoperative fibrinogen level.

(4) What's the ASA score?

Answer: According to American Society of Anesthesiologists (ASA), ASA score of all participators were 1 in 198 cases and 2 in 57 cases by referring to the collected material once more.

(5) Any pt operated for obstruction?

Answer: There were no cases who received operation for obstruction, because this situation easily caused emergency. As I told the above, emergency patients were excluded in the study. We excluded about 26 cases because of the reason.

(6) What was the operative evaluation and was metastatic disease ruled out ?

Answer: Our preoperative evaluation mainly included disease status evaluation and preoperative tolerance evaluation. For preoperative tolerance evaluation, it included organic function evaluation of heart, kidney, lung, liver, brain, et al, Immune status, drug allergy situation, nutritional status, et al. According to related examination results, we decided whether it was a proper participator in the study.

For disease evaluation, we used Colonoscopy, Pathological examination, Immunohistochemistry, Siemens 64-slice spiral CT, MRI, type-B ultrasonic of regional lymph nodes, et al.

Facing uncertain diagnosis of metastasis, we used enhanced CT, PETCT (all types of uncertain micrometastases),

MRI(some micrometastases, uncertain property lumps, especially in liver and abdominal/pelvic lymph node metastasis), Puncture biopsy, et al.

All patients with metastasis were ruled out.

(7) Does all stage II received chemotherapy?

Answer: Not all the patients with stage II received chemotherapy. In stage II patients, only those with high-risk factors received chemotherapy, including poor differentiation, large lumps, T4, tumor resection margin was less than 1cm, fewer than 12 lymph nodes for postoperative biopsy.

(8) Type of recurrence (distal vs local)?

Answer: The total recurrence patients were 124 cases, in which local recurrence patients were 36 cases and distant metastasis patients were 88 cases.

(9) Abbreviations in the abstract

Answer: I had supplemented full names of Abbreviations.

Reviewer code: 00180872

(1) As for innovation

Answer: There have been some published literatures on relationship of fibrinogen and malignancies. However, most of them are about some other types of malignancies, such as esophagus cancer, cervical cancer, ovarian cancer, endometrial cancer, lung cancer, and so on, while there are very few literatures on colon cancer. From the study, we primarily investigated detection significance of preoperative fibrinogen on patients with colon cancer. It was confirmed preoperative fibrinogen level was negatively relevant to prognosis, especially discuss on different stage patients.

(2) There was little information provided about the 255 patients enrolled in this study. how were they diagnosed, treated, and followed? What were preformed prior to operation to determine a “metastatic free” condition?

Answer: For not enough information, I supplemented some necessary information, such as gender, ASA score, chemotherapy group of stage II and III, exclusion criteria situation, detection method, et al. Some other detailed information in Table1.

For preoperative evaluation, before operation, we made disease status evaluation and preoperative tolerance evaluation. For operative tolerance evaluation, it included organic function evaluation of heart, kidney, lung, liver, brain, et al, Immune status, drug allergy situation, nutritional status, et al. According to related examination results, we decided whether it was a proper participator in the study. For disease evaluation, we used Colonoscopy, Pathological examination, Immunohistochemistry, Siemens 64-slice spiral CT, MRI, type-B ultrasonic of regional lymph nodes, et al. Facing uncertain diagnosis of metastasis, we used enhanced CT, PETCT (all types of uncertain micrometastases), MRI(some micrometastases, uncertain property lumps, especially in liver and abdominal/pelvic lymph node metastasis), Puncture biopsy, et al. All patients with metastasis were ruled out.

For therapy, all participators were performed radical surgery. Stage II patients with high-risk factors (including poor differentiation, large lumps, T4 stage, less than 1cm of tumor resection margin, fewer than 12 lymph nodes for postoperative biopsy) and Stage III patients received postoperative adjuvant chemotherapy.

For follow-up, after surgery, all the patients were followed up once every 3 months within 2 years and then once every 6 months within from 2 years to 5 years, with postoperative 5 years as the deadline. Two follow-up ways were used, outpatient or inpatient review and telephone follow-up.

(3) Is there a uniform diagnosis and treatment guideline in the institute? Did the patients receive any adjuvant treatment after surgery?

Answer: For uniform diagnosis, I have mentioned above(question 2).

For treatment guideline, I have mention some(question 2). Firstly, all received colon cancer radical surgery. Then for some stage II and the whole stage III patients received adjuvant chemotherapy scheme FOLFOX6 as followings, oxaliplatin (L-OHP) injection, 130mg/m², intravenously infused for 3 hours, on the first day, calcium folinate (CF) injection, 300mg/m², intravenously infused, on the first day, 5-FU injection, 400 mg/m², intravenously injected, on the

first day, and 5-FU 2400 mg/m², continuously intravenously infusion by micro pump for 48 hours. 14 days a cycle, 12 cycles in total. For the patients with drug resistance, chemotherapy scheme XELOX was applied, as followings, oxaliplatin (L-OHP) injection, 130mg/m², intravenously infused for 3 hours, on the first day, Capecitabine tablets, 1000 mg/m², po., twice a day, from first day to fourth day, 3 weeks a cycle.

(4) What were the pathology diagnoses, all adenocarcinoma? Since the pathology subtypes might influence the fibrinogen level, the authors need to clarify this

Answer: No. In the study, I used only Grade(tumor cell differentiation degree), including adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma. In the study, only Grade was referred as pathology types. In table 1, Grade was not associated with fibrinogen level(P=0.08). Thus, I did not discuss more.

(5) The authors excluded some patients (no number provided) from analysis for several pre-existing confounding factors, like infection, hematology disease, ect. However there were nearly one third of patients in the study group had smoking habit. Since smoking were known to have impact on the serum fibrinogen level, as well as clinical outcome, the author need to explain why or why not include this group of patient in analysis. Furthermore, the result should be adjusted with smoking.

Answer: Smoking were showed to be associated with serum fibrinogen level(P<0.05). I supplemented some discussion content. Such as: As for smoking effect on fibrinogen and malignancy, Charles[24] reported that smoking was a high risk of malignancies(HR:2.12, 95% CI:1.16-3.84, P=0.01) by an adjusted Cox regression of 14,320 participants, and was closely related with serum fibrinogen level. And participants who had ceased smoking for a mean of 11-18 years had 4-10% lower fibrinogen levels than their current smoking counterparts. The above was in accordance with our study. Nevertheless, its detailed mechanism needed further studies. As for the result, through reading more literatures again, I think it may be unnecessary to adjusted with smoking. However, I supplemented some new discussion content.

(6) The author use mean +/- SD to describe fibrinogen level (which were usually used in a parametric data). Since the study numbers were quite small, it would be relatively rare to be presented in a normal distribution.

Answer: I used mean \pm SD to describe fibrinogen level according to some literatures, such as Son HJ, et al. *Ann Surg Oncol* 2013;20:2908-2913; Ji Won Park, et al. *Ann Surg Oncol* (2010) 17:2839-2846, et al. You are right: The larger the quantity is, the better it will be. However, I consulted statistical expert and was told it was enough although it was not so large as you mentioned.

(7) As the authors demonstrated in table 2, there were 77% of patients had fibrinogen lower than 2.61 g/L, the “mean of 3.17 \pm 0.88” should be revised.

Answer: In the table 2, about the number 77% in Fibrinogen(<2.61) really means that 5-y DFS was 77% for the patients with Fibrinogen(<2.61), meanwhile 5-y DFS was 36.9% for the patients with Fibrinogen(\geq 2.61).

Actually, you can know from the table 2 that patients with Fibrinogen(<2.61) were 74 cases(32.89%) and patients with Fibrinogen(\geq 2.61) were 181 cases(67.11%). Thus, fibrinogen level mean was 3.17 \pm 0.88.

(8) If the authors would like to claim the prognostic value of pre-operative fibrinogen, they should include multivariate Cox analysis to evaluate the prognostic value between so many factors, like TNM, CEA, mGPS, WBC, NLR, PLR and fibrinogen.

Answer: Your suggestion is very helpful and I have added the related analysis content in my paper, such as, “Prognostic value were ordinally fibrinogen, TNM stage, mGPS, AFP and CEA for 5-y DFS, and fibrinogen, mGPS, TNM stage, AFP and CEA for 5-y OS (Table 3 in detail).”

(9) In this study, the author used a cut-off-point as 2.61 g/L, which was actually much lower than previous studies. The authors need to explain this in the discussion section.

Answer: Higher than 2.61 g/L of fibrinogen as a cut-off-point might be in more literatures on some other malignancies or diseases. However, some literatures of gastrointestinal malignancies or diseases used 2.61 g/L of fibrinogen as a cut-off-point, such as Son HJ, et al. *Ann Surg Oncol* 2013;20:2908-2913, et al, in some of which participants were Asian people. In addition, I used the cut-off in the study and got similar fibrinogen effect on colon cancer. Thus, it was proper to be used in our study.

(10) Furthermore since the patients enrolled were of great variety, the author should also clarify the prognostic impact of fibrinogen in each stage of patients. Was the results the same in patients with stage 1 and stage 3 disease?

Answer: Thank you very for this suggest and I have added detailed related content of prognostic impact of fibrinogen in each stage of patients into our paper. It can be found in abstract, results, discussion, et al.

(11) Did this study review by an independent ethics committee? The authors should address this in their manuscript.

Answer: The research was approved ethically by Ethics Committee of Tumor Hospital of Xinjiang Medical University (No.W-201305). All patients provided informed written consent, which can be found in MATERIALS AND METHODS.

(12) In the abstract section, there were too many abbreviations and the authors need to clarify them before using these in scientific writing.

Answer: I have added their full names in abstract section.

(13) The discussion was underdeveloped.

Answer: According to the suggestions of four reviewers, I modified related discussion content.

Reviewer code: 00068230

(1) The language of paper is very poor and there are number of grammatical/spelling errors in the text.

Answer: With the help of AMEuc company, our paper was modified much and there was no errors left with language modified proof.

(2) The methodology portion is too short e.g. it does not indicate what was the exact number of patients selected initially for study before exclusion of some patients.

Answers: We added related content in the paper. Exclusion criteria: using procoagulant or anticoagulant drugs within 8 weeks (11 cases) , combined with hepatitis(23 cases), kidney disease(9 cases), myocardial infarction(14 cases), hypertension(47 cases), other infectious or hematologic diseases which could influence fibrinogen level(22 cases). Facing uncertain diagnosis of metastasis, we used enhanced CT, MRI(some micrometastases, uncertain property lumps, especially in liver and abdominal/pelvic lymph node metastasis), PETCT (all types of uncertain micrometastases), Puncture biopsy.

(3) What was the exact day used to collect the blood samples prior to surgery.

Answer: 5~7 days before operation.

(4) What were the other various reasons due to which 30 cases were lost during follow up etc etc.

Answer: Main reason was losing contact them. 11 cases changed their residences and their telephones. 2 cases went abroad. 3 cases got dysthymia and rejected to cooperate with us. 7 cases changed hospital because of bad family circumstances. 4 cases stopped continuous treatment because of not enough treatment fee. 1 cases committed suicide because of insupportableness for the disease. 2 cases were not known about their reasons.

(5) Although authors have reported number of tables but results have been written very briefly as well as poorly.

Answer: Your suggestion is very helpful and I supplemented some detailed results into RESULT section.

(6) There is a similar literature published.

Answer: There have been some published literatures on relationship of fibrinogen and malignancies. However, most of them are about some other types of malignancies, such as esophagus cancer, cervical cancer, ovarian cancer, endometrial cancer, lung cancer, and so on, while there are very few literatures on colon cancer. Although there is a very similar paper, in my paper I explored preoperative fibrinogen prognostic effect not only in total patients, but also in each stage: Stage I, Stage II and Stage III. It was confirmed preoperative fibrinogen level was negatively relevant to prognosis. Thus, it is promising to be an independent prognostic factor for the patients with colon cancer in the future by more studies confirming this.

(7) In order to add weightage to the submitted manuscript, some mechanistic studies involving cause of increase in fibronogen content during colon cancer malignancy is needed.

Answer: Your suggestion is very helpful for us. Undoubted, I did not performed some mechanism of fibrinogen as a prognosis. However, I discussed some mechanism on it, such as the followings: First of all, fibrinogen multiple

integration and non-integrin receptors are found in tumor cells, stromal cells and inflammatory cells. The interaction between fibrinogen mediated by specific receptors and cells can control cell proliferation, migration, apoptosis and expression of signaling molecules and inflammatory mediators. What's more, fibrinogen and combination of growth factors of vascular endothelial growth factor(VEGF), fibroblast growth factor(FGF) and so on, can stimulate angiogenesis. The process consequently regulate endothelial cell proliferation and angiogenesis^[17,18]. Thus, they play an important role in angiogenesis^[19]. And fibrinolytic system originating from fibrinogen also plays a facilitating role in angiogenesis and proliferation process of tumor cells^[20]. they can enhance adhesion between tumor cells and platelets or endothelial cell to promote blood transfer ^[21]. In addition, fibrinogen was ever confirmed to be an important determinant factor in metastatic potential of circulating tumor cells in study by mice lung cancer models of fibrinogen-deficient^[22]. And fibrinogen can eliminate tumor cells by hindering Natural Killer cell-mediated, consequently to increase their metastatic potential^[23]. The above suggests that fibrinogen is involved in tumor cell invasion and metastasis.

Your very significant suggestions will help us to design deeper experiment about their mechanism of fibrinogen function in patients with colon cancer. Thank you very much again for your advice.

Reviewer code: 00074923

(1) I would like a better explanation of the demographic data: gender, comorbidities, ASA grade. Specially for stage III, if any difference in terms of adjuvant therapies was found between groups.

Answer: According to American Society of Anesthesiologists (ASA), ASA score of all participants were 1 in 198 cases and 2 in 57 cases. As for gender, male/female was 135/120. Exclusion criteria: metastasis(39 cases), using procoagulant or anticoagulant drugs within 8 weeks(11 cases), combined with hepatitis(23 cases), kidney disease(9 cases), myocardial infarction(14 cases), hypertension(47 cases), other infectious or hematologic diseases which could influence fibrinogen level(22 cases).

For some stage II with high risk factors and the whole stage III patients received adjuvant chemotherapy scheme FOLFOX6. For the patients with drug resistance, chemotherapy schemeXELOX was applied. In stage III patients, there were obvious significant difference of 5-y OS and 5-y DFS between hyperfibrinogen group and hypofibrinogen group (5-y OS:P=0.037, 5-y DFS:P=0.025, Figure 1D). To know whether fibrinogen level can influence chemotherapy in stage III patients need deeper experiment. However, through our rough observation of drug resistance and side reaction during treatment, no difference in terms of adjuvant therapies was found between groups.

(2) Please explain why you use the 2.61 cuts off of fibrinogen levels.

Answer: Higher than 2.61 g/L of fibrinogen as a cut-off-point might be in more literatures on some other malignancies or diseases. However, some literatures of gastrointestinal malignancies or diseases used 2.61 g/L of fibrinogen as a cut-off-point, such as Son HJ, et al. *Ann Surg Oncol* 2013;20:2908–2913, et al, in some of which participants were Asian people. In addition, I used the cut-off in the study and got similar fibrinogen effect on colon cancer. Thus, it was proper to be used in our study.

(3) I would like you to clarify the impact of fibrinogenos in prognosis by each TNM stage. Is still statistically significant when adjusted by each stage?

Answer: We did statistical analysis for each stage patients, as following: Kaplan-Meier curves showed that for the whole patients, both 5-y OS and 5-y DFS in hypofibrinogen group were much higher than in hyperfibrinogen group (5-y OS:P=0.000, 5-y DFS:P=0.000, Figure 1A); For patients with stage II and III, there were obvious significant difference of 5-y OS and 5-y DFS between hyperfibrinogen group and hypofibrinogen group (5-y OS:P=0.000, 5-y DFS:P=0.000, Figure 1C; 5-y OS:P=0.037, 5-y DFS:P=0.025, Figure 1D); but not within stage I patients(5-y OS:P=0.688, 5-y DFS:P=0.384, Figure 1B) maybe because of no enough quantity, which was not as large as stage II or stage III and needs to enlarge sample size for more precise conclusion, for the whole patients showed difference of prognostic effect of preoperative fibrinogen. From above, preoperative fibrinogen could be a independent prognostic factor in stage II, III, or the whole patients.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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