Editorial Office,
World Journal of Gastroenterology
August, 3th, 2019

Dear Editor,

Re: Manuscript ID: 49701
Please find an attached revised version of our manuscript "Post-transplant Infection Improves Outcome of Hepatocellular Carcinoma Patients after Orthotopic Liver Transplantation", which we would like to resubmit for publication in World Journal of Gastroenterology.

The comments made by reviewers are highly insightful, which enable us to greatly improve the quality of our manuscript. In the following pages are our point-to-point responses to each of the comments suggested by the reviewers.

We hope that the revisions in the manuscript and our responses to the comments will make our manuscript suitable for publication in World Journal of Gastroenterology.

We look forward to hearing from you at your earliest convenience.

Yours sincerely,
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Responses to the comments of Reviewer No. 03479537

1. In the methods, the patient inclusion for transplant is defined by several well-known transplant criteria. It is possible that a patient is admitted to receive liver transplantation based on one or another of these criteria. It is not possible that the same patient will meet all the criteria (the authors used and in their description) as the inherited difference among the different criteria necessitates that if the patient meets one, will not meet the other at the same time.

Response: We thank the reviewer for this comment. As we described in our manuscript, we gradually expanded the selection criteria for transplant recipients with HCC (Milan Criteria – UCSF Criteria – Hangzhou Criteria) from 2002. From August 2002 to November 2006, patients with HCC that met the Milan Criteria were included for orthtopic liver transplantation (OLT). From December 2006 to May 2008, patients who met the UCSF Criteria were selected for OLT. And from June 2008 to date, Hangzhou Criteria were used for patient enrollment in our transplant center. Therefore, one patient needs to fulfill only one of these Criteria in a specific period of time, not all of them. To make our presentation more clear, we would like to replace "… and Hangzhou Criteria …" with "… or Hangzhou Criteria …" as shown in the revised manuscript.

2. The survival figures given for patients with PTI per group need revising as the DFS is longer than OS at 3 and five years and should match the figure1.

Response: Thanks for this comment. We reconfirmed the authenticity and accuracy of our data and consulted our statistician. We realized that the confusion lies in the improper definition of the events for recurrence-free survival (RFS). We took "detection of tumor relapse after OLT" as an event for RFS, but improperly excluded "death without tumor recurrence" as an event for RFS. In this situation, RFS of 3 and 5 years were higher than overall survival (OS) of our cohort. Therefore, we would like to modify the definition of the events for RFS as "detection of tumor relapse after OLT" or "death without tumor recurrence after OLT". The 1-, 3-, 5-year OS rates of the study cohort were 86.6%, 69.0%, 63.6%, and 1-, 3-, 5-year RFS rates were 75.7%, 60.0%, 57.3%, respectively. We accordingly revised the manuscript, the RFS survival curves in Figure 2C and 2D were also revised.

3. It is not clear why the authors provided a cutoff value for αFP of 200. None of the criteria declared in the methods utilized this cutoff value as a prognostically determinant element.

Response: We thank the reviewer for this comment. Large body of evidence supported the prognostic relevance of preoperative α-fetoprotein (AFP) level in transplant HCC patients with HCC. For example, AFP at a cutoff value of 200 ng/mL has almost perfect specificity (99–100%) in HCC diagnosis (Semin Liver Dis. 2019 May;39(2):163-177). Preoperative AFP level > 200 ng/mL independently predicts post-transplant tumor recurrence within 2 years (Liver Transpl. 2007 Mar;13(3):391-9). Moreover, the combination of the preoperative AFP level (cut-off value is 200 ng/mL) and positron emission tomography data predicts better outcomes than those using the Milan criteria in living donor liver transplantation (J Hepatol. 2016 Apr;64(4):852-9). Therefore, a cutoff value of 200 ng/mL for preoperative AFP level is of prognostic value, which is accepted by the transplant community.

4. Some studies have identified a relationship between the grade of infection and the OS after cancer treatment. Others have linked the localization of infection to the latter parameter. Could the authors try to assertain any of the findings? Due to the sensitivity of the topic, we suggest that the authors enrich their discussion with the counterargument.

Response: We thank the reviewer for this comment. In our study, we defined post-transplant infection (PTI) by referring to the international sepsis forum consensus conference on definitions of infection, as briefly described in the attachment number 1. That is, PTI was diagnosed by a panel of experts when the patient had septic symptoms and met the PTI definition. Actually, most of the patients enrolled in the PTI group experienced significant clinical manifestations of systemic inflammatory reaction, which indicated that the infection grade was severe. Nevertheless, the grade of infection was hard to be quantified and defined because of lacking a clear and widely accepted judging criterion. We assume that PTI that reach the level of sepsis may be able to improve patient prognosis after orthotopic liver transplantation (OLT). In Discussion section, we mentioned the study reported by Bohman et al. (Neurosurgery. 2009;64:828-834). In that study, they compared 17 glioblastoma patients who suffered postoperative infection with 51 matched patients without infection, but failed to find a surmised correlation between postoperative infection and prolonged lifetime, although subgroup analysis of patients with deep infection showed a longer survival trend, indicating that the severity of infection may impact on survival of glioblastoma patients. In another study, Bonis et al. analyzed 197 glioblastoma patients treated by surgical resection, among whom 10 patients experienced post-operative infection, and 8 had a deep infection. The results showed significant association between post-operative infection and prolonged survival (Neurosurgery 2011; 69:864-868). The discrepancy between the two studies may be attributed to differential diagnostic criteria of post-operative infection, because infection occurred less frequently and more severely in the later study. Thus, it can be deduced that probably only severe infection exerted potent anti-tumor effects and improved patient outcomes. The rationale may reside in whether the severity of microbial infection reaches the level or degree to stimulate whole body immune system and evoke a cascade of cytokines that may destroy the residual tumor cells. In our cohort, 53 patients were diagnosed PTI and the localization of infection included lung, abdomen, urinary tract, intravascular catheter, incision and bloodstream (Supplementary Table 2). Considering that the origination (infection sits) of PTI is distant from the primary tumor location (the liver) and different from the post-transplant tumor recurrence sites, we speculated that the tumor suppressive function of PTI might be mediated by activating the whole body immune system, not by regional immune mechanism. Therefore, we speculate that the prognostic effect of PTI was independent of the infection sites. Due to the relative small number of the PTI group, it is not appropriate to subdivide the patients according to the localization of infection and ascertain their prognostic impact respectively. Further multicentre studies (to increase the number of patients) are needed to validate our findings and clarify whether infection localization differentially impacts on patient prognosis or not. We revised the discussion section according to the reviewer's valuable comment.

Responses to the comments of Reviewer No. 00077376

1. In the abstract, there are no data on exact number of patients analyzed, including PTI and non-PTI groups. This is mandatory.

Response: Thanks for this comment. A total of 238 patients were enrolled in our study, including 53 patients belonged to the PTI group and 185 patients belonged to the non-PTI group. We accordingly revised the abstract as shown in the manuscript.

2. The exact follow-up schedule should be described to determine recurrence-free survival. For example, enhanced CT scan after operation is performed every 3 months until one year, and every 6 months thereafter.

Response: We thank the reviewer for this comment. The transplant patients with HCC were followed up at the outpatient clinic according to the standard protocol of our center, as we described in our previous paper published in Cancer Letters with modifications (Cancer Lett. 2011 Jul 28;306(2):214-22). Briefly, tumor recurrence was monitored by measurements of plasma AFP and/or abnormal prothrombin and abdominal ultrasonography (every month the first half year, then every 3 months), lipiodol computed tomography (every 3 months for the first year, every 6 months for the second year and once per year thereafter). Magnetic resonance imaging, positron emission tomography or radioisotope bone scan were taken when necessary. We accordingly revised the manuscript.

3. According to the data shown in Table 1, the incidence of vascular invasion was very high, reaching over 50% of the recipient in each group. Major vascular invasion was contraindication for LT, and thus this high incidence means that microscopic vascular invasion was very high. Is this correct? You should explain the reason why the incidence of microvascular invasion was very high, compared with those of the previous reports.

Response: Thanks for this comment. In our cohort, 197 out of 238 patients have definite description of vascular invasion (VI) status of the tumor in their pathology reports. We failed to acquire the information of VI status in pathology reports of the left 41 patients. The deficiency of the VI information could be explained by following reasons: (1) The pathologist of our hospital lacks diagnostic experience during the initial period of liver transplantation (between the years 2002-2003); (2) Preoperative treatment such as TACE and ablation caused tumor necrosis in some patients which made the made the diagnosis of VI difficult; (3) VI was not found by the examiner and IV status was omitted in pathological reports, which was the most common case. We, therefore, speculated that most of the 41 patients had tumors actually without VI. With respect for data accuracy, we excluded these 41 patients in the VI related statistical analysis. Among the 105 patients with VI on explant pathology, 99 patients were diagnosed with microvascular invasion, and 6 patients with macrovascular invasion in the distal branches of portal vein or hepatic veins explored by pathological examination. The diagnosis of macrovascular invasion was only made postoperatively, not by imaging examination in the preoperative setting. In our study, VI was used to describe tumors with microvascular invasion or macrovascular invasion, such definition of VI was accepted by the transplant community and reported in previous literature (HPB (Oxford). 2018 Aug;20(8):768-775).

4. In Table 1, there are no data on HCV virus infection. Are there any patients with HCV virus infection?

Response: We thank the reviewer for this comment. In China, most of the HCC patients (approximately 90%) have the background of liver cirrhosis caused by chronic infection of hepatitis B virus (HBV), not by hepatitis C virus (HCV) (Front Oncol. 2019 May 21;9:370). In our cohort, 214 (89.9%) patients had positive HBsAg, 24 patients (10.1%) showed negative HBsAg. Among the 24 HBsAg negative patients, 5 patients (2.1%) have HCV, 5 (2.1%) have alcoholic disease, 10 (4.2%) have autoimmune liver disease and 4 (1.7%) have cryptogenic cirrhosis.

5. How do you explain the reason why early postoperative event of infectious compilation after LT highly influence the survival benefit even when tumor recurrence occurs. What kind of factors do you imagine or speculate?

Response: Our data demonstrated that PTI improves overall survival rate of transplant HCC patients. Subgroup analyses revealed that PTI remarkably improved OS (p=0.003) and RFS (p=0.003) rates of

HCC patients with vascular invasion (IV), but did not impact on OS (p=0.404) and RFS (p=0.304) of patients without VI. Theoretically, VI represents invasion of HCC cells into vasculature of the liver and indicates escapement of HCC cells from primary tumor into circulation. In the patients without VI, excellent long-term survival free from tumor recurrence could be achieved by surgical procedure alone, because liver transplantation (LT) offers the chance of complete removal of tumor cells. In patients with VI, although LT could maximally remove the primary tumor, the residual circulating HCC cells may represent the main source of tumor recurrence after LT. Therefore, the reason why PTI improves OS and RFS of patients with VI may reside in the inhibitory effect of PTI on the residual circulating HCC cells. We speculate that PTI stimulates systematic immune response to eliminate the residual HCC cells, probably via activating innate immunity, the first line of defense from infection, and adaptive immunity that has characteristics of specific killing ability and immunological memory. The effect of PTI on eliminating tumor cells might be mediated by early reactions of innate immunity and later responses of adaptive immunity, for example, the efficacy of antigen presenting cells boosts the killing power of $CD8^+$ T cells and evokes a cascade of cytokines that enrich and strengthen immune cells, to eliminate tumor cells in the circulation even when tumor recurrence occurs. The underlying antitumor mechanism of infection-inducing immune stimulation is unclear (J Immunother Cancer. 2018 Aug 6;6(1):78), further investigation to elucidate the underlying mechanisms is urgently needed.

Responses to the comments of Reviewer No. 02444864

1. Line 128: "pathogens" instead of "pathogen". Line 129: "However" should be omitted. Lines 149 and 152: lymph nodes (two words). Lines 157 and 181: "absence of" instead "without".

Response: Thanks for this comment. We have accordingly revised the manuscript.

2. Lines 204-206: The virus-related carcinogenesis doesn't have to be mentioned. Thus "likewise" (line 207) should be also deleted.

Response: We thank the reviewer for this comment. We revised the manuscript in the discussion portion according to reviewer's suggestion.

3. Line 251-253: the possible role of innate immunity should be mentioned.

Response: Thanks for this comment. We have revised the manuscript in the discussion portion according to the reviewer's suggestion.

Responses to the comments of Reviewer No. 00054275

1. Line 130: 3 patients from the 8 with negative microbiological cultures, were diagnosed with CMV infection; which type of PTI was present in the remaining 5 patients?

Response: Thanks for the comment. In the PTI subgroup, negative microbiological cultures were found in 8 patients, among whom 3 patients were diagnosed with cytomegalovirus infection by whole- blood CMV DNA measurement. Microbiological cultures were drawn in the left 5 patients probably due to the use of broad-spectrum antibiotics. Because bacteria are the most common pathogens that cause PTI, we speculate that these 5 patients were probably infected by certain kinds of bacteria.

2. Lines 140-141: overall survival 3 and 5 years post LT seems to be less than recurrence free survival but obviously it cannot be.

Response: Thanks for this comment. The comment is also suggested by Reviewer No. 03479537

(comment 2). We have redefined the “events” for recurrence-free survival (RFS), and accordingly revised the manuscript.

3. Line 151: what does mean "cirrhotic background"? Perhaps seriousness of cirrhosis...?
Response: Thanks for this comment. Cirrhotic background means the background of liver cirrhosis. Liver cirrhosis is deemed as premalignant condition for liver cancer. Most of patients developed liver cancer in the background of liver cirrhosis, but a small proportion of patients developed HCC without cirrhotic background. In this study, 214 patients have HCC developed on the background of liver cirrhosis, 24 patients have HCC without liver cirrhosis.

4. Is there any relation between type of infection (bacterial or fungal, or site of infection...) and HCC recurrence?
Response: We thank the reviewer for this comment.

We demonstrated that PTI significantly improved OS and RFS of transplant HCC patients with VI, regardless of the type of pathogen. As shown in Supplementary Table 3, PTI was predominated by bacterial infections followed by fungal and viral infections. Due to the limited case of the PTI group, we did not subdivide PTI group according to the type of pathogens and ascertain their impact on survival respectively. Previous study revealed better control of lung tumor challenge in the influenza experienced mice than the infection naïve control via enhancement of cancer immunosurveillance (Cancer Immunol Res 2014, 2:263-273). Therefore, we speculate that both virus and bacteria could stimulate the immune system and then amplify the desired anti-tumor responses, clearing distant tumor cells and preventing recurrence of the cancer. Nevertheless, further studies with larger patient number may be needed to clarify whether type of pathogen impacts on prognosis of transplant HCC patients.

In our cohort, 53 patients were diagnosed PTI and the localization of infection included lung, abdomen, urinary tract, intravascular catheter, incision and bloodstream (Supplementary Table 2). The origination (infection site) of PTI is distant from the primary location of the tumor (the liver), and is different from the common post-transplant tumor recurrence sites (liver, lung, bone, brain, and adrenal gland). Therefore, we speculated that PTI activated the whole body immune system, not regional immune mechanism for its anti-tumor function, and the origination (infection site) of PTI may not influence on HCC recurrence after liver transplantation. Due to the relative small number of the PTI group, it is not suitable to subdivide the patients according to the infection origination and ascertain their prognostic impact respectively. Further multicentre studies to increase the number of patients are needed to clarify whether infection site differentially impacts on patient prognosis or not.

5. Possibly immunosuppression therapy may have been reduced in case of PTI and it may influence HCC recurrence: an evaluation of this point should be reported and discussed.
Response: We thank the reviewer for this comment. We have standard protocol for the use of immunosuppressive agents in our center as was briefly described in the manuscript. During the first month after transplantation, the dose and choice of drugs were individually different, frequent change of the dose of immunosuppressive agents was necessary in order to adjust blood concentration of the drug in a relatively low and safe range (e.g. tacrolimus 7-9 ng/ml). This range of FK506 is relatively lower than that of the patients in Western countries. Because high exposure to immunosuppression agents may promote tumor recurrence after liver transplantation, minimal immunosuppressive suppression strategy was applied for all the HCC patients in our center. When the patient show signs of post-transplant

infection (PTI), transient reduction or withdrawal of the immunosuppressive agents was considered, but as soon as PTI was controlled the drugs were added or reused to avoid acute rejections. Because the median duration of PTI in our cohort is 12 days (IQR: 7.0 to 21.75 days), the duration of the patients with PTI exposed to low immunosuppressive agents was usually short. The influence of reduction or withdrawal of immunosuppressive agents on HCC recurrence was not easy to be accessed in the PTI patients for the following reasons: 1. not every patient with PTI was given a reduced dose of immunosuppressive drugs, and the extent of drug reduction (including the dose and time) was quite different between individuals; 2. The category and regimen of immunosuppressive drugs changed during the past twenty years. Our results demonstrated that PTI improves overall and recurrence-free survival of transplant HCC patients with vascular invasion (VI) but not of those without VI, suggesting that the prognostic benefits of PTI may reside in inhibiting HCC progression after liver transplantation. We further showed that the benefits of PTI lasted even after tumor recurrence, because the post-recurrence survival (PRS) of the patients who suffered post-transplant tumor relapse was significantly improved. These findings suggested a sustained anti-cancer effect of PTI which could not simply explained by transient reduction or withdrawal of immunosuppressive agents during PTI period. Therefore, we speculate that PTI stimulates systematic immune response to eliminate the residual HCC cells, probably via activating innate and adaptive immunity that has characteristics of specific killing ability and immunological memory. The effect of PTI on eliminating tumor cells might be mediated by the early reactions of innate immunity and the later responses of adaptive immunity, for example, the efficacy of antigen presenting cells boosts the killing power of $CD8^+$ T cells and evokes a cascade of cytokines that enrich and strengthen immune cells, to eliminate tumor cells in the circulation even when tumor recurrence occurs. The underlying antitumor mechanism of infection-inducing immune stimulation is unclear (J Immunother Cancer. 2018 Aug 6;6(1):78), further investigation to elucidate the underlying mechanisms is urgently needed.

6. Which was the aetiology of cirrhosis before LT? Is there any relation between etiology of cirrhosis and HCC recurrence?

Response: thanks for this comment. In our cohort, 214 patients have liver cirrhosis, 202 (94.4%) patients have HBV-related liver cirrhosis, 5 (2.1%) patients have HCV-related liver cirrhosis, 4 (2.0%) patients have alcoholic liver disease, 2 (1.0%) patients have autoimmune liver disease and 1 (0.5%) patient has unknown etiology. Most of the patients in our cohort have HBV-related liver cirrhosis, other etiology accounts for only a very small portion of the whole cohort. Therefore, it is not desirable to subdivide the group according to the cirrhotic etiology and analyze the relation between cirrhotic etiology and HCC recurrence.

Responses to the comments of Reviewer No. 00182703

1.The article is very interesting and useful. There are numerous cases of neoplasms that have evolved favorably after infections with different germs and different localizations. The authors of this article had the excellent idea of studying the evolution of HHC patients undergoing OLT with and without post-transplant infection. The group of patients with post-OLT infection is not very large, but the results are statistically significant. The material and method are very clearly presented. The statistical analysis is correct, as well as the interpretation of the results and discussions with references to literature data. Further studies are needed to confirm the results and to find the clear pathophysiological mechanisms that intervene and make the evolution of the infected patients to be favorable.

Response: We thank the reviewer for this comment. Post-transplant infection (PTI), a common complication, is deemed to be harmful for the liver transplant recipients from a short-term perspective. However, the present study provided a novel conception that PTI was not simply a harmful event for liver transplant recipients, but a factor could exert significant overall (OS) and recurrence-free survival (RFS) benefits for the HCC recipients with high risk of post-OLT death and tumor recurrence, which may be attributed to the tumor suppressive effect conferred by PTI. Moreover, improved OS and PRS in the patients who suffered HCC recurrence with PTI compared with those without PTI suggested persistent tumor suppressive effect of PTI on the patient even after tumor relapse. Just as suggested by the reviewer, before moving forward, our data needs to be verified prospectively in larger cohorts and the mechanism by which PTI inhibited HCC progression should be investigated in well-designed studies. Our findings may stimulate successful management of post-transplant HCC recurrence in the future.

Responses to the comments of Reviewer No. 00051373

1. This manuscript is not well written.

Response: We thank the reviewer for this comment. This manuscript is written by senior doctors and edited by MedSci which offers standard services in paper evaluation and language editing. We have made our every effort to write and revise the manuscript.

2. Too many citations in the section of methods. It is not a good written in this section.

Response: We thank the reviewer for this comment. The definition of PTI is very important and relatively complex, concerning the six most frequent causes (pneumonia, intra-abdominal infection, urinary tract infection, surgical wound infection, intravascular catheter-related infection and bloodstream infection), we briefly explained in the attachment 1 due to the word and space limits of the paper. Furthermore, we cited the previous literature according to the consensus reached by international sepsis forum regarding the definition of infection in order to support our study. In this paper, we chose to evaluate PTI that occurred with 30 days following OLT mostly during ICU stay for the following two reasons: 1.the recipients had the highest incidence of PTI during the first month following OLT; 2. the records of patients' data were more reliable in the ICU settings. We cited the reference 13, 17 and 18 to support the setting of the post-transplant time limit of PTI (30 days). We believed that these citations in the "Study design and patients" section are necessary and valuable to support our study. Likewise, in the "Peri-transplant management" and "Immunosuppression and post-transplant follow-up" sections, the citations are all necessary and important to support the present study.

3.It seems to be a chart record or report of the paper reading.

Response: We thank the reviewer for this comment. This study is a retrospective cohort study, we extracted infection-related information and clinicopathological parameters from the chart record and/or electronic medical record system of our hospital. This is the basic methodology for a retrospective cohort study.

4. The topic is the post transplantation infection, which should be included not only in bacteria but also in viral infection particular in HBV and HCV.

Response: We thank the reviewer for this comment. As we clearly described in the "materials and methods" section, PTI definition is briefly presented in the attachment number 1 and described in details in the reference 16. The pathogens of PTI in this study included bacteria (Klebsiella pneumoniae, Acinetobacter baumannii, enterococcus faecalis, stenotrophomonas maltophilia, etc.), fungus (Candida albicans, Candida prapsilosis, Aspergillus, etc.) and virus (cytomegalovirus). HBV and HCV are important etiologies of chronic liver disease, especially HBV in current study, they were commonly eradicated after

liver transplantation. In a small portion of recipients, recurrence of HBV or HCV may occur months or years later, but almost impossible within 30 days after OLT. Besides, HBV and HCV recurrence seldom causes acute inflammation in the liver recipients, which obviously did not meet the definition of PTI. Therefore, HBV and HCV infection clearly not belonged to PTI category.

5.Infection category in the table is too compact and making a confusion

Response: We thank the reviewer for this comment. In Supplementary Table 3, the summarized microbiological cultures result of the PTI patient group. The pathogen of PTI was predominated by bacterial infections followed by fungal and viral infections. We demonstrated that PTI significantly improved OS and RFS of transplant HCC patients with VI, regardless of the type of pathogen. We speculate that all kinds of pathogens, including bacteria, fungus and virus could stimulate the immune system and then amplify the desired anti-tumor responses, clearing distant tumor cells and preventing recurrence of the cancer. Therefore, more detailed division of infection category should not be necessary and not helpful for the following statistical analyses. Nevertheless. further studies with larger patient number may be needed to clarify whether type of pathogen, e.g. bacterial, fungal and/or viral, impacts on prognosis of transplant HCC patients.