Dear Editor,

We really appreciate the comments and suggestions you and the reviewers made on our manuscript "***High mortality associated with Gram-negative bacterial bloodstream infection in liver transplant recipients undergoing immunosuppression reduction***", which was submitted for possible publication in the journal of World Journal of Gastroenterology.

We have made careful modification to the manuscript according to the comments and suggestions. A point-to-point response is also attached below for your reference.
If any other information is required, please contact us at qianyb79@hotmail.com.

Best regards.

Yours sincerely,
Yongbing Qian

Point-to-point response to Reviewers' Comments

Reviewer: 1

Comment 1:

The title needs to be rewritten, in its current form it confusing, not informative nor attractive to the great audience.

Response:

We really appreciate your advice. The title was changed to "High mortality associated with Gram-negative bacterial bloodstream infection in liver transplant recipients undergoing immunosuppression reduction".

Comment 2:

In the abstract, the aim of the study is not clear and does not make any sense. A study willing to summarize something is not acceptable. It is mandatory to clarify in the abstract the term "immunosuppression reduction" whenever it is first used in the text.

Response:

We have carefully revised the abstract accordingly. The following text is added or modified.

Background

Update the content as "Immunosuppression is an important factor in the incidence of infections in transplant recipient. Few studies are available on the management of immunosuppression (IS) treatment in the liver transplant (LT) recipients complicated with infection. The aim of this study is to describe our experience in the management of immunosuppression treatment during bacterial bloodstream infection (BSI) in LT

recipients, and assess the effect of temporary immunosuppression withdrawal on 30-day mortality of recipients presenting with severe infection." (**Line 39-45**)

Result

Modify the term of "immunosuppression reduction" as: immunosuppression reduction (at least 50% dose reduction or cessation of one or more immunosuppressive agent). (**Line 55-56**)

Add the content "Cox regression showed that rejection (aHR 7.021, *P* = 0.001) and complete IS withdrawal (aHR 12.65, *P* = 0.019) were independent risk factors for 30-day mortality in patients with GNB infections after LT." (**Line 64-66**)

Comment 3:

Introduction is quite confusing and needs to be improved. Authors must clarify the definition and use of immunosuppression reduction besides literature supporting its efficacy and/or effects on the infection/transplant outcome. This information must be followed by the types of immunosuppressive drugs most frequently used and dosage.

Response

The specific regimens are added. The corresponding text is modified, which is now updated as follows:

The commonly used immunosuppressive agents after LT include calcineurin inhibitor (CNI) such as tacrolimus (0.1-0.15 mg/kg/day in 2 doses) or ciclosporin (6-8 mg/kg/day in 2 doses), mycophenolate mofetil (500-1000 mg, bid), sirolimus (2 mg/day), and corticosteroids (induction with high dose methylprednisolone 500-1000 mg IV, followed by tapering over 5 days to maintenance with prednisone 5-20 mg/day). It is highly controversial regarding the management of IS therapy during infection after LT, although IS reduction (partially discontinue or reduce the dosage of at least one immunosuppression agent) or complete withdrawal may be a generally accepted option in life-threatening infections. To date, only few studies assessed the impact of IS reduction or complete withdrawal of immunosuppressive therapy on infection outcomes in LT recipients [8, 9]. In these studies, researchers reported that immunosuppressive agents may be discontinued completely in kidney transplant recipients since hemodialysis is an effective option in case of rejection. In contrast, complete discontinuation of immunosuppression is highly dangerous in liver transplantation because it may lead to graft loss and patient death. (**Line 92-103**)

Comment 4:

The criteria for inclusion/exclusion must be better described.

Response:

The criteria for inclusion/exclusion described as follows:

All the enrolled LT recipients satisfied the inclusion criteria: 1) 18 to 75 years of age; and 2) with diagnosis of bloodstream infection confirmed by blood culture. The

patients were excluded if infection was localized or in the brain, or patients died on the day of surgery. (**Line 118-121**)

Comment 5:

The topic - Antimicrobial prophylaxis is confusing and needs to be improved. How frequently was the colonization with MRSA screened? Also, authors have described: "Alternative regimen including vancomycin may be considered for the patients with a history of MRSA infection or colonization". Considering the study is already done, how the authors stated that vancomycin could probably be considered???? The same observation for "…surgeon may modify…". In fact, which protocol was used? Authors need to be more specific.

Response:

In our center, patients undergoing transplant surgery were routinely screened for MRSA nasal colonization, when the patient was included on transplant waiting list and transferred to liver intensive care unit after the operation. The physician adjusted the prophylactic use of antibiotics according to the pathophysiological conditions of patients before surgery and a history of MRSA infection or colonization.

The corresponding modified information is added to the text as follows: Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization was routinely screened, when the patient was included on transplant waiting list and transferred to liver intensive care unit after the operation. Alternative regimen including vancomycin may be considered for the patients with a history of MRSA infection or colonization. The surgeon may modify the prophylactic regimen according to the history of infectious disease based on the experience of our center. (**Line 134-139**)

Comment 6:

Authors must describe the sequences (forward and reverse) of the primers used in the study.

Response:

The specific regimens are added to the text, which is now updated as follows: including: bla_{KPC}-related sequences (5'-TCTGGACCGCTGGGAGCTGG-3', forward and 5'-TGCCCGTTGACGCCCAATCC-3', reverse); bla_{OXA}-23-related sequences (5'-GATCGGATTGGAGAACCAGA-3', forward and 5'-ATTTCTGACCGCATTTCCAT-3', reverse), and bla_{NDM}-related sequences 5'-GGTTTGGCGATCTGGTTTTC-3', forward and 5'-CGGAATGGCTCATCACGATC-3', reverse). (**Line 176-181**)

Comment 7:

Authors must describe which were the doses used before and after immunosuppression reduction. Also, it is necessary to state which was the drug discontinued in another patient.

Response:

Thank you for your suggestion. We have updated the doses used before and after immunosuppression reduction. You can see the statements about the doses of immunosuppression before reduction in **lines 92-97,** and the statements of immunosuppression doses after reduction was showed in **lines 190-192**. We also changed the content "the drug discontinued in another patient" to "the drug discontinued in one patient". (**Line 255**)

Comment 8:

The conclusion needs to be improved.

Response:

The conclusion is updated accordingly as follows:

In conclusion, IS reduction is surprisingly more common in case of GNB than GPB BSIs in the LT recipients. MDR GNB infection may put LT recipients at higher risk of graft rejection and death than GPB infection. Rejection and complete IS withdrawal are the independent predictors for the 30-day mortality in patients with GNB infection. Complete IS withdrawal should be cautious due to increased risk of mortality in the LT recipients complicated with GNB infection. A multidisciplinary approach, timely and appropriate successful antimicrobial therapy, and source control, when necessary, may be safe and more effective than IS reduction therapy in recipients with BSI after LT. (**Line 376-382**)

Reviewer #2:

Comment 1:

In the abstract, the content of methods is inadequate because the subjects and examination methods are not described concretely.

Response:

The corresponding information is added to the text:

Abstract > Methods

All recipients diagnosed with BSI infections after LT were included in this study. Univariate and multivariate Cox regression analysis of risk factors for 30-day mortality was conducted in LT patients with GNB infections. (**Line 49-51**)

Comment 2:

In the abstract, immunosuppressive and its abbreviation IS are not used properly.

Response:

Thank you for your reminding. We have modified these contents properly.

Comment 3:

In the abstract, the mortality rate should be described according to the two group, GPB and GNB groups, showing the statistical difference.

Response:

The corresponding information is added to the text as follows:

Abstract > Results

The mortality rate in GNB group (39.3%, 11/28) was significantly higher than that in GPB group (4.8%, 2/42) (P = 0.001). All the deaths in GNB group were attributed to worsening infection secondary to IS withdrawal but the deaths in GPB group were all due to graft-versus-host-disease. (**Line 59-62**)

Comment 4:

I am very surprised with the fact that your institution had performed LT for 1297 recipients during only two years. This means that nearly 2 LT procedures per day had been performed in a single institution. Among them, 786 recipients are children. What percentage of these patients had living donor? The exact number of living donor and deceased donor should be described.

Response:

Thank you for your compliment to the liver transplantation center of our hospital. The number of LT ranked Number one in last 5 years in China. Among the children's liver transplantation, the exact number of living donor and deceased donor was 650 cases and 136 cases, respectively. The specific content has been added to the text. (**Line 117**)

Comment 5:

The median (IQR) time from the day of transplantation (day 0) to onset of BSI was 4 (1-6) days in GPB group (n = 45) and 12 (8-41) days in GNB group (n = 29). Are this data significantly different?

Response:

The data of the median (IQR) time from the day of transplantation (day 0) to onset of BSI was significantly different between the two groups. We have added this data to Table 1.

Comment 6:

Table 1 shows that the rates of intra-abdominal infection, IS reduction including complete withdrawal and death were significantly higher in GNB group, compared to GPB group. However, these findings are not mentioned in the section of results. Furthermore, you should discuss these points more clearly.

Response:

Thank you for your suggestion. We have added the corresponding content to the text.

Abstract > Results:

GNB group was associated with significantly higher incidence of intra-abdominal infection, IS reduction, and complete IS withdrawal than GPB group (P < 0.05). (**Line 62-64**)

Discussion

Add the contents: "The deaths were more likely associated with epidemiologic and

technical-surgical factors." (**Line 317-318**) and "Reduction of biliary complications was thought to be an important factor for lower incidence of bacteremia, especially in living-donor liver transplantation because biliary tract is one of the most common port of bacterial entry due to the complexity of liver transplantation procedures [2]. Similar to previous reports [36-38], the primary site of infection was not identified in 17.6% of the cases in this study, probably due to early proactive antibiotic therapy and the difficulty of identifying intra-abdominal and biliary sources." (**Line 357-363**)

Comment 7:

IS reduction was found in 28 (41.2%) cases, specifically 5 cases (5/28, 17.9%) in GPB group and 23 cases in GNB group. Is this significantly different? It seems that IS reduction rate was significantly higher in GNB group.

Response:

Significant difference in IS reduction was found between the GPB group and GNB group, which was shown in Table 1.

Science editor

Comment 1:

(1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

Response:

Thank you for your reminding. All the materials will be submitted.

Comment 2:

(2) I found the authors did not add the PMID and DOI in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout;

Response:

We have modified the format of the references according to the requirements of the journal.

Comment 3:

(3) I found the authors did not write the "article highlight" section. Please write the "article highlights" section at the end of the main text.

Response:

The corresponding statements is added to the text as follows:

ARTICLE HIGHLIGHTS

Research background

Bacterial infections continue to be the most common infectious complication after liver transplantation (LT), usually within 2 months after LT. Immunosuppression (IS) is the single most important factor contributing to the incidence of infections in

transplant recipients.

Research motivation

It is highly controversial regarding the management of IS therapy during infection after LT, although IS reduction (partially discontinue or reduce the dosage of at least one immunosuppression agent) or complete withdrawal may be a generally accepted option in life-threatening infections. Few studies are available on the management of IS treatment in the LT recipients complicated with infection.

Research objectives

To describe our experience in the management of immunosuppression treatment during BSI in LT recipients, and assess the effect of temporary immunosuppression withdrawal on 30-day mortality of recipients presenting with severe infection.

Research methods

A retrospective study was conducted with the patients diagnosed with BSI after LT in Department of Liver Surgery, Renji Hospital from January 1, 2016 through December 31, 2017. All recipients diagnosed with BSI infections after LT were included in this study. Univariate and multivariate Cox regression analysis of risk factors for 30-day mortality was conducted in LT patients with GNB infections.

Research results

Seventy-four episodes of BSI were identified in 70 LT recipients, including 45 episodes of gram-positive bacterial (GPB) infections in 42 patients and 29 episodes gram-negative bacterial (GNB) infections in 28 patients. Overall, immunosuppression reduction (at least 50% dose reduction or cessation of one or more immunosuppressive agent) was made in 28 (41.2%) cases, specifically, in 5 (11.9%) cases with GPB infections and 23 (82.1%) cases with GNB infection. The 180-day all-cause mortality rate was 18.5% (13/70). The mortality rate in GNB group (39.3%, 11/28) was significantly higher than that in GPB group (4.8%, 2/42) ($P = 0.001$). All the deaths in GNB group were attributed to worsening infection secondary to IS withdrawal but the deaths in GPB group were all due to graft-versus-host-disease. GNB group was associated with significantly higher incidence of intra-abdominal infection, IS reduction, and complete IS withdrawal than GPB group ($P < 0.05$). Cox regression showed that rejection (aHR 7.021, $P = 0.001$) and complete IS withdrawal (aHR 12.65, $P = 0.019$) were independent risk factors for 30-day mortality in patients with GNB infections after LT.

Research conclusions

Immunosuppression reduction is more frequently associated with GNB infection than GPB infection in LT recipients. Complete IS withdrawal should be cautious due to increased risk of mortality in the LT recipients complicated with BSI.

Research perspectives

IS reduction may be a generally accepted option in life-threatening infections after LT.

However, this practice must be put into heated discussion, as it seems to be associated with worse outcome in patients with BSI. A multidisciplinary approach, timely and appropriate successful antimicrobial therapy, and source control, when necessary, may be safer and more effective than IS reduction therapy in recipients with BSI after LT. (**Line 384-431**)

Comment 4:

(4) Re-Review: Required.

Response:

According to your helpful suggestions, our manuscript has been further reviewed by a microbiologist and an expert in Infectious Diseases.