

ANSWERING REVIEWERS

January 8, 2015



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15465-Review.doc).

Title: Sequential *vs* simultaneous revascularization in patients undergoing liver transplantation: A Meta-Analysis

Author: Jia-Zhong Wang, Yang Liu, Jin-Long Wang, Le Lu, Ya-Fei Zhang, Hong-Wei Lu, Yi-Ming Li

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 15465

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

1 Format has been updated

Response: Thanks for the reviewers' and editors' kindly suggestions. These comments are all valuable and very helpful for revising and improving our paper. We have studied comments carefully and have made corrections which we hope meet with approval. Revised portion are highlighted in the paper. Firstly, our manuscript has been modified by the language professional English language editing company, which was recommend by *World Journal of Gastroenterology* and could provide language certificate letter. Secondly, we added some parts to our manuscript according to the editors' kindly suggestions, such as Conflict-of-interest, Data sharing, Core tip, and COMMENTS. Thank you for your suggestions concerning our manuscript ([ESPS Manuscript NO: 15465](#)). Thirdly, we thoroughly checked the manuscript to make sure that all of the references were properly cited; and one of my references had no PMID and DOI, because this reference was very important for our research, we decided to cite the reference. Finally, all authors checked and revised the manuscript according to the Format for meta-analysis, The Revision Policies of BPG for Meta-Analysis and other documents.

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

(1) Reviewer #1: Well written paper analyzing a hot topic in liver transplantation technique and its outcomes: However, further data should be mentioned in the analysis or in the discussion: 1. rate of patients with hepatic artery thrombosis as an early postoperative complication.

Response: Thanks for your kindly suggestions! Thrombosis of the hepatic vasculature presents late after liver transplantation in fewer than 5% of patients (Late Surgical Complications Following Liver Transplantation, Paige M. Porrett, 2009). Despite the low incidence of these complications, they are associated with substantial morbidity and may require retransplantation. Therefore, we added the data that the rate of patients with hepatic artery thrombosis resulted in retransplantation as an early postoperative complication (Page11, Line11-12). According to the included studies of our manuscript, vascular complications were absent except for one case of hepatic artery thrombosis leading to retransplantation in SeqR (Page11, Line12-13). Among the late vascular complications seen in liver transplant recipients, hepatic artery thrombosis is the most common. Although early HAT may present in a fulminant manner, the presentation of late hepatic artery thrombosis is often very indolent. Approximately half of patients who present with late hepatic artery thrombosis will be asymptomatic (Delayed hepatic artery thrombosis in adult orthotopic liver transplantation: a 12-year experience, Bhattacharjya S, 2001), and the thrombosis will be discovered during routine ultrasonographic evaluation of the hepatic vasculature. These patients can be managed conservatively but expectantly, as 25% to 30% will develop biliary strictures or graft failure and require retransplantation.

Reviewer #1: causes of graft failure with a special focus on ischemic cholangitis.

Response: Thanks for your valuable suggestions! Liver ischemia-reperfusion injury during transplantation involves necessarily the peribiliary plexus resulting in endothelial cell activation, which may lead to microvascular thrombosis, microcirculatory disturbances and again ischemia. Stricture formation, biliary apoptosis, necrosis, and cholangitis are the results and may even lead to progressive graft failure. Indeed, it seems that cholangiocytes are more sensible to the ischemic insult than the liver parenchyma (Ischemia-Reperfusion Injury and Ischemic-Type Biliary Lesions following Liver Transplantation, Raffaele Cursio, 2011). Therefore; we improve our manuscript (Page11, Line13-15).

Reviewer #1: rate of retransplantations owing to ischemic graft injury, acute or chronic.

Response: Thanks for your kindly suggestions! Some studies demonstrated that chronic or chronic graft injuries were risk factors for development of ITBLs following LT. Polak et al reported that

primary nonfunction was diagnosed in 3 % (SeqR) versus 1 % (SimR) and the rate of retransplantation was 9% in SeqR and 7% in SimR, respectively. We added the rate of retransplantations (Page11, Line10-12). Although approximately 75% of the total liver blood flow is provided by the portal vein, the hepatic artery supplies approximately 50% of the oxygen consumed by the liver in physiologic conditions. In SeqR, the graft is exclusively perfused through the portal vein for at least 10 min until the realization of the hepatic arterial anastomosis. SimR elicits a remarkable improvement in oxygen tension and maintenance of tissue ATP, compared to sequential revascularization. The disadvantage of SimR is the prolongation of warm ischemia time and the anhepatic phase, which can be detrimental to postoperative graft function and survival (Influence of warm ischemia time on initial graft function in human liver transplantation, K. P. Platz).

(2) Reviewer #2: This work is a meta-analysis concerning the aspect of sequential versus simultaneous portal and arterial reperfusion in liver transplantation. The authors performed a structured literature review with finally analysis of six studies including overall 467 patients. Like expected in patients with simultaneous reperfusion a significant longer warm ischemic time was found. In contrast ischemic-type biliary lesions were significant reduced in the group of patients with simultaneous reperfusion. Graft failure and mortality were not different between both groups at one month and one year after liver transplantation. A disadvantage of the work is the short follow-up period of the included studies (RCT Baccarani et al median follow-up of 17/19 month, RCT Adani et al median follow-up 13/14 month) and therefore the analysis of only the short term graft and patient survival and the short term biliary complications. Comparison of sequential and simultaneous reperfusion showed a significant prolonged warm ischemic time in patients with simultaneous reperfusion, this could possibly result in a reduced long-term graft survival or increased late biliary complication.

Response: Thanks for your kindly suggestions! You are right that our work is the short follow-up period of the included studies. No consensus exists regarding the most optimal sequence of revascularization of the liver graft during liver transplantation. The disadvantage of SimR is prolongation of the warm ischemia time and the anhepatic phase, which can be detrimental to postoperative graft function, survival, and morbidity. In the clinical setting, the most commonly used procedure for revascularization of the liver graft is initial reperfusion via the portal vein and subsequent reconstruction of the hepatic artery. This is based on the experience that portal blood flow alone is sufficient for adequate hepatocellular function. However, the main disadvantage of IPR might

be an increased risk of warm ischemic damage to the bile ducts, which depend solely on arterial blood supply. It is also well known that biliary epithelial cells are more susceptible to warm ischemia and reperfusion injury than hepatocytes. Which revascularization results in a better graft and patient survival remains unclear? This is the reason that we undertook this meta-analysis to compare the relationship between revascularization and graft and patient survival. According to the results of our work, the kind of revascularization did not affect the graft and patient survival after LT in short follow-up period. However, there was not enough data to analyze the relationship between revascularization and graft and patient survival in long-term follow-up period. Randomized, blinded and controlled clinical trials with large sample size are needed to study the relationship between revascularization and graft and patient survival in long-term follow-up period.

(3) Reviewer #3: Interesting meta-analysis, nice work. Suggestion: 1) maybe add a paragraph in the conclusion to discuss the impact of donor factors on ITBLs.

Response: Thanks for your kindly suggestions! Yes, there are lots of donor factors affecting the development of ITBLs. Therefore, we chose some important donor factors, which were organized in a paragraph (Page11, Line22-26). In the past, liver transplantation across ABO blood group barriers has been discouraged because of multiple complications, particularly acute rejection and biliary complications. However, organ shortage and new developed immunosuppressive agents decreasing humoral rejection have led to an increased use of ABO-incompatible liver for transplantation with acceptable results concerning patient and graft survival rate. The incidence of ITBLs after ABO-incompatible LT in adults is much higher than in ABO-compatible LT (Recipient outcomes after ABO-incompatible liver transplantation: a systematic review and meta-analysis, 2011). Gender-mismatched liver transplant recipients had a higher likelihood of graft failure when compared with gender-matched liver transplant recipients. Moreover a female to male donor/recipient match is associated with late occurrence of ITBLs. Loss-of-function mutation in the CC chemokine receptor 5 has been associated with development of ITBLs following LT (chemokine receptor 5 Δ 32 polymorphism—a risk factor for ischemic-type biliary lesions following orthotopic liver transplantation, 2004). In a multivariate logistic regression analysis the donor age was found to be independent risk factors for ITBL ($p < 0.001$ respectively) (Ischemic-type biliary lesions after liver transplantation: a retrospective analysis of risk factors and outcome, 2014). The presence of the MMP-2 CT genotype in donor and/or recipient was found to increase the incidence of ITBLs incidence stepwise from 9% when absent, increasing to 16% when present in either donor or recipient, further increasing to 29% when present in

both donor and recipient (Matrix metalloproteinase 2 genotype is associated with nonanastomotic biliary strictures after orthotopic liver transplantation). Since lots of factors had an impact of donor factors on ITBLs, we added a paragraph in the conclusion. Thank you again for your constructive suggestions!

Reviewer #3: Most of the included patient in this study dated before 2006. During the last decade more high risk livers are transplanted, does simultaneous revascularization is also beneficent for this patient group? Or do these liver grafts benefit from sequential revascularization to reduce warm ischemia time?

Response: Thanks for your kindly suggestions! You are right that most of the included patient in this study dated before 2006 and only two studies were published after 2010 (Contemporaneous Portal-Arterial Reperfusion during Liver Transplantation: Preliminary Results, 2011; Protection of the intrahepatic biliary tree by contemporaneous portal and arterial reperfusion: results of a prospective randomized pilot study, 2012). In one study, the LT was always carried out by piggy-back technique in all cases. The bile duct was reconstructed in all cases by termino-terminal duct to duct anastomosis; the T-tube was used, respectively, in 32% versus 29% of cases. All grafts were whole liver except one extended right liver split including segment one in SeqR group. Intrahepatic nonanastomotic biliary strictures in 26% versus none ($P = 0.01$), respectively, in SeqR and SimR. Innovative and exciting advances in the clinical science in liver transplantation continuously realize as the results of studies, clinical trials, international conferences, consensus conferences, new technologies and discoveries. The percentage of all donors who are Standard Criteria Donors (SCD) is on the decline and there is an increase in Expanded Criteria Donors (ECD). Improving the organ cold storage by machine perfusion (MP) has been proposed to improve the solid organ outcomes, especially in liver (What's new in clinical solid organ transplantation by 2013, Maurizio Salvadori, 2014). Unfortunately, there were no studies conducted to compare the relationship between revascularization and graft, patient survival in long-term follow-up period in recent years. Randomized, blinded and controlled clinical trials with large sample size are needed to study the relationship between revascularization and graft and patient survival. However, the arterial warm ischemia time in SeqR was statistically significant longer than warm ischemia time in the SimR group confirming a possible greater amount of selective arterial ischemia to the bile duct in the group with sequential portal and arterial revascularization. This resulted in a higher incidence of nonanastomotic intrahepatic biliary strictures in the SeqR group (26% versus none) thereby suggesting a possible protective role of SimR on the integrity of the intrahepatic

biliary tree potentially due to less selective arterial ischemia. However, whether SimR or SeqR is beneficent for the patients with more high risk livers remain unclear. More clinical studies are needed to be conducted.

3 References and typesetting were corrected

Thanks so much for the editors' overwhelming suggestions and patience! We corrected the references of our work using ENDNOTE(X6). Only one reference did not have PMID and DOI, and we will submit the first page of the paper. Our manuscript has been modified by the language professional English language editing company, which was recommend by *World Journal of Gastroenterology* and could provide language certificate letter. We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in highlighted in revised paper. We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.

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

Sincerely yours,

A handwritten signature in black ink, appearing to be 'Li Yi-Ming' in a stylized cursive script.

Yi-Ming Li,

Department of General Surgery,

Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine,

Xi'an, China 710004.

E-mail: wjz05202156@stu.xjtu.edu.cn;

Tel: +8613992836340;

Fax: +8687679746.