ANSWERING REVIEWERS

November 8, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5475-review.doc).

Title: Consecutive laparoscopic gallbladder and spleen resection for liver cirrhotic patients

Authors: Ming-Jun Wang, Jun-Li Li, Jin Zhou, Zhong Wu, Bing Peng

Name of Journal: *World Journal of Gastroenterology*

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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 reviewers

3 Point-by-point responses to each of the reviewers' comments are provided below (in red)

4 References and typesetting have been corrected

If you any questions, please do not hesitate to contact us. Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely,

Bing Peng, MD, PhD
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We would first like to express our sincere gratitude to the reviewers for their constructive and positive comments.

Comments

Replies to Reviewer 1:

Comments: This is an interesting study. The authors should justify why splenectomy was indicated - in general, this should be avoided in cirrhotic patients. More details should be given: how were patients selected and how was treatment decided. Were all eligible patients included, if not more details should be given? Were complications, blood use, hospital stay, long term follow-up, pain etc assessed in a prospective way, if not, the outcomes must be less confident than stated. More details should be given about the surgeon's expertise and possible learning effect.

(1) The authors should justify why splenectomy was indicated - in general, this should be avoided in cirrhotic patients.

Indeed, splenectomy was once considered contraindicated for hypersplenism secondary to liver cirrhosis; however, this recommendation was mainly based upon the technical difficulties associated with splenomegaly, well-developed collateral vessels, and increased risk of bleeding. The technical improvements in laparoscopic technique and accumulation of surgeons' experience have led clinicians to question this concept in recent years. Moreover, several recent studies have demonstrated the promising results for the short- and long-term outcomes of splenectomy treatment in cirrhosis patients with hypersplenism. In Japan, splenectomy is a common intervention used to improve thrombocytopenia resulting from hypersplenism in cirrhotic patients[1-3], and the procedure has been demonstrated to improve liver fibrosis and liver function[4, 5]. In addition, clinical studies and systemic reviews have indicated that laparoscopic splenectomy is a well-tolerated surgical procedure with good surgical and immune function outcomes[6-9]. On the other hand, very few studies of splenectomy treatment of hypersplenism have been reported from western countries, and transcatheter partial splenic arterial embolization appears to be the preferred treatment option for hypersplenism due to liver cirrhosis. These differences in treatment practices may due to the particular health care policies, medical levels, and clinical decisions of the different countries. Admittedly, there is much data in the publicly available literature to support the notion that splenectomy (by both open and laparoscopic approaches) is a safe, feasible, and effective procedure for treating hypersplenism secondary to liver cirrhosis. Animal experiments have also demonstrated that splenectomy can enhance liver regeneration in the cirrhotic context, and shown that it exerts a preventive role against CCl_4-induced liver fibrosis[10-12]. Thus, we considered that splenectomy was indicated for the patients who met the indications mentioned in our manuscript.

(2) More details should be given: how were patients selected and how was treatment decided.

We apologize for this oversight in detailing the strategies used for patient selection and treatment decision. The appropriate information, including the inclusion and exclusion criteria, has been presented in Figure 1 and in the text of the Materials and Methods section. Specifically, the following information regarding treatment decision was clarified as: "Indications for splenectomy were as follows: (1) severe thrombocytopenia due to hypersplenism with a platelet count of $<30\times10^9$/L and/or a white blood cell count of $<3\times10^9$/L; (2) being prone to oesophageal variceal haemorrhage as a result of severe portal hypertension."

From a mechanistic perspective, splenectomy decreases portal vein pressure and reduces platelet destruction, thereby reducing the risk of oesophageal variceal haemorrhage and improving thrombocytopenia. A previous study using a rat liver fibrosis model indicated that

spleen-derived TGF-β1 is involved in the development of liver fibrosis; the splenectomy-mediated decrease in TGF-β1 level is expected to improve liver fibrosis by inhibiting hepatic stellate cell activation[13]. As stated in our response to comment (1), splenectomy is a common intervention used in Japan to improve thrombocytopenia resulting from hypersplenism in cirrhotic patients[1-3], and this procedure has been demonstrated to improve liver fibrosis and liver function[4, 5]. In addition, clinical studies and systemic reviews have indicated that laparoscopic splenectomy is a well-tolerated surgical procedure with good surgical and immune function outcomes[6-9]. Animal experiments have also demonstrated that splenectomy can enhance liver regeneration in the cirrhotic context, and shown that it exerts a preventive role against CCl_4-induced liver fibrosis[10-12]. Thus, based on the results of these studies, we considered that splenectomy was indicated for our enrolled patients (i.e. the treatment decision was established).

(3) Were all eligible patients included, if not more details should be given?
As stated in comment (2), the inclusion and exclusion criteria have been presented in Figure 1 and in the appropriate text of the Materials and Methods section. According to the criteria, all eligible patients were included for further analyses.

(4) Were complications, blood use, hospital stay, long term follow-up, pain etc assessed in a prospective way, if not, the outcomes must be less confident than stated.
Our study was retrospective and subject to the well-characterized potential biases related to this type of study design. However, the preoperative information was not significantly different among the three treatment groups (Table 1), which could balance the potential biases to some extent. On the other hand, our single-centre study design limited the amount of study participants (liver cirrhosis patients with hypersplenism and gallstones receiving LC plus LS) available for analysis. Over the 10-year period examined (2003-2013), only 17 candidates fit the study inclusion criteria. It will take much time and dedicated funding to perform a prospective randomized clinical trial (RCT) for this topic; even so, a large-volume, prospective RCT is absolutely necessary to confirm our results. We hope to secure the funding and collaborations necessary to carry out such a study in the future.

(5) More details should be given about the surgeon's expertise and possible learning effect.
We recently published a study related to these exact topics: The learning curve for laparoscopic splenectomy for massive splenomegaly: a single surgeon's experience. *Chin Med J* (Engl); 2013[14]. The related information has been cited and described in the Materials and Methods sub-section of Surgical techniques. Specifically, all of the operations were performed by a single team led by a chief physician who is an experienced expert at LS. The surgeon's expertise and learning curve are presented in detail in referenced publication.

Replies to Reviewer 2:

Comments: Why performing a splenectomy? Despite the mentioned indications for splenectomy in the study, none of the labs cutoff were related to its clinical implications. Absolute numbers should not be an indication for surgery and most centers won't perform a splenectomy for the cited reasons. Also, were those standard indications in the clinical service? If not, what happen to the

other patients with same values that were not taken to the operating room? - The authors are putting too much emphasis comparing the laparoscopic and the open cholecystectomy/splenectomy groups to point out difference that are expected. The study should focus more on the fact that minor/not significant differences were found between the LS versus the LC/LS groups. - Due to the small sample size, the authors cannot confirm that there is no difference in portal/splenic vein thrombosis between groups. - It seems really unlikely a 3 minute difference between LC+LS compared to the LS group. As pointed out by the authors, performing a cholecystectomy in patients with cirrhosis requires additional time to avoid bleeding during dissection plus the change in positioning of the patient, so how the authors explained this? Were the more skilled surgeons performing the LC/LS and the less skilled surgeons the LS alone? - In table 3, authors reported that all the patients who underwent LC/LS and almost all the patients who underwent LS became Child A class. How this happen? Performing a splenectomy don't change any of the child class (and that is confirmed by the authors as bilirubin levels were almost unchanged while albumin levels decrease) so how this happen?

(1) Why performing a splenectomy? Despite the mentioned indications for splenectomy in the study, none of the labs cutoff were related to its clinical implications. Absolute numbers should not be an indication for surgery and most centers won't perform a splenectomy for the cited reasons. Also, were those standard indications in the clinical service? If not, what happen to the other patients with same values that were not taken to the operating room?

Indeed, none of labs cutoff values were considered as clinical indicators of selecting splenectomy for the liver cirrhosis patients. In western countries, splenectomy is not the preferred treatment method for liver cirrhosis patients with hypersplenism and/or portal hypertension, and transcatheter partial splenic arterial embolization (TPSAE) is used instead. However, a primary disadvantage of TPSAE is the risk of serious complications, which range from severe pain to pancreatitis, atelectasis and microcoil migration. It is undeniable that splenectomy was once considered contraindicated for treating hypersplenism secondary to liver cirrhosis; this recommendation was mainly based upon the technical difficulties associated with splenomegaly, well-developed collateral vessels, and increased risk of bleeding. The technical improvements in laparoscopic technique and accumulation of surgeons' experience have led clinicians to question this concept in recent years. From a mechanistic perspective, splenectomy decreases portal vein pressure and reduces platelet destruction, thereby reducing the risk of oesophageal variceal haemorrhage and improving thrombocytopenia. A previous study using a rat liver fibrosis model indicated that spleen-derived TGF-β1 is involved in the development of liver fibrosis; the splenectomy-mediated decrease in TGF-β1 level is expected to improve liver fibrosis by inhibiting hepatic stellate cell activation[13]. Moreover, several recent studies have demonstrated the promising results for the short- and long-term outcomes of splenectomy treatment in cirrhosis patients with hypersplenism. In Japan, splenectomy is a common intervention used to improve thrombocytopenia resulting from hypersplenism in cirrhotic patients[1-3], and the procedure has been demonstrated to improve liver fibrosis and liver function[4, 5]. In addition, clinical studies and systemic reviews have indicated that laparoscopic splenectomy is a well-tolerated surgical procedure with good surgical and immune function outcomes[6-9]. Therefore, splenectomy was performed for our patients if they met the indications mentioned in our manuscript. For patients who did not meet the selection criteria, endoscopic variceal ligation, endoscopic injection sclerotherapy, platelet transfusion, or TIPS were selected as the alternative intervention.

(2) The authors are putting too much emphasis comparing the laparoscopic and the open cholecystectomy/splenectomy groups to point out difference that are expected. The study should focus more on the fact that minor/not significant differences were found between the LS versus the LC/LS groups.

We chose to emphasize the comparison of the laparoscopic and open cholecystectomy/splenectomy groups in our original manuscript as this was the purpose of our study. We apologize for the confusion as to why we did not instead emphasize the minor/non-significant differences observed between the LS group vs. the LC/LS group. Our primary objective of comparing the outcomes between the laparoscopic and the open cholecystectomy/splenectomy groups was chosen because of the great improvements in the laparoscopic technique that have been introduced in recent years and the patients' preference for receiving the less invasive laparoscopic surgeries when possible. We added Group 2 (patients treated with LS alone) in order to investigate whether the LC plus LS consecutive procedure may be less well-tolerated by the patients (i.e. whether the increased extent of operative manipulation may worsen the surgical outcomes as compared with LS alone). We found that almost all outcomes were comparable between the two laparoscopy-treated groups; however, a higher incidence of PSVT (11.8%) was observed in the LC plus LS treated patients when compared with either the patients treated with LS alone (6.9%) or those treated with open surgery (7.1%). Unfortunately, the small sample size precluded our ability to confirm that concomitant laparoscopic cholecystectomy and splenectomy was a significant risk factor of PSVT. Therefore, if comparison with Group 3 (traditional open surgery) proved that consecutive LC plus LS is a feasible, safe, and effective procedure for treating liver cirrhosis patients with gallstones and hypersplenism from one perspective, then Group 2 proved it from another perspective.

(3) Due to the small sample size, the authors cannot confirm that there is no difference in portal/splenic vein thrombosis between groups.

We apologize for our inaccurate description regarding the incidence of PSVT observed between the treatment groups in our study. Indeed, our small sample size precluded our ability to confirm that there is no difference in PSVT between the groups. To clarify this issue, the original statement has been revised as follows: "The rate of PSVT was higher in Group 1 (11.8%) than in Group 2 (6.9%) and Group 3 (7.1%), but the small sample size precluded our ability to determine whether concomitant LC and LS was a significant risk factor of this complication."

(4) It seems really unlikely a 3 minute difference between LC+LS compared to the LS group. As pointed out by the authors, performing a cholecystectomy in patients with cirrhosis requires additional time to avoid bleeding during dissection plus the change in positioning of the patient, so how the authors explained this? Were the more skilled surgeons performing the LC/LS and the less skilled surgeons the LS alone?

We agree that many readers may suspect this outcome. To clarify this issue, we have carefully re-checked all of our data and analyses; the results were all the same as stated in the original manuscript. In addition, we clarified in the manuscript that all of the operations were performed by a single team with the same lead surgeon. It is well accepted that surgeons need

a steep learning curve period to be adapt to laparoscopic surgeries. Thus, during the early period of the learning curve it will take more time to perform LS even if the spleen is normal or smaller immune thrombocytopenic purpura (ITP) is present. When performing LS for liver cirrhosis patients with enlarged spleens, more attention should be paid in order to avoid injuring the pancreas tail and the well-developed collateral vessels near the splenic hilum; during the learning curve period, LC plus LS is not routinely considered. After the learning curve has been achieved, however, LC plus LS can be considered. Thus, the distribution of LS and LC plus LS was unbalanced based on the learning curve period. The time spent on LC during the late stage of the learning curve will be partially balanced by the time spent during the early period of the learning curve.

It is also important to note that nearly 1000 LCs were performed in our hospital over the past 10 years by the lead surgeon of this study. A professional team of anaesthesiologists and nursing staff also contributed partially to saving time when performing LS plus LC. Because the physician's learning curve was not the theme of this study, we did not mention this information in detail. However, to clarify this issue we have referenced our recently published study on this exact topic (The learning curve for laparoscopic splenectomy for massive splenomegaly: a single surgeon's experience. *Chin Med J* (Engl); 2013[14]).

(5) In table 3, authors reported that all the patients who underwent LC/LS and almost all the patients who underwent LS became Child A class. How this happen? Performing a splenectomy don't change any of the child class (and that is confirmed by the authors as bilirubin levels were almost unchanged while albumin levels decrease) so how this happen?

The changes of the Child-Pugh class presented in Table 3 were determined from the data collected over a follow-up period of 6 months after the laparoscopic or open surgeries. Initially, we had planned to show the long-term results of LC plus LS vs. LS alone and the open consecutive procedures; however, considering that the follow-up time was not long enough we had to focus on the perioperative outcomes. Unfortunately, we accidentally left some data in Table 3 from our original attempt to perform analysis on the long-term changes of the Child-Pugh class. These data have been deleted from the revised manuscript.

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