Peer Review File

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Reviewer A

To the authors of the manuscript, I have found the article interesting and the information you provide fruitful for future research.

Comment 1: In the Results section you should explain with more detail the serum differences between SHAM, 70%Ph and 90%Ph. I would also include other serum markers to further prove that what you are observing is a readout of the liver health.

Reply 1: Thank you very much for reviewing our manuscript and your comment. As your comment, we added a detailed description of the biochemical analysis in the results section as attached. In terms of serum markers to reflect the functional status of liver, biochemical parameters analyzed in this study are optimal up to date in a clinical setting. AST and ALT are good indicators of ongoing hepatocyte injury. TBIL and PT-INR, which are currently usually used to diagnose PHLF, reflect liver dysfunction. We added a detailed meaning of the biochemical parameter for the evaluation of liver function in the discussion as follows.

(Biochemical analysis after liver resection in Results)

Serial changes of AST, ALT, TBIL, and PT-INR observed in three groups at PO, 1, 6, 14, 22, 30, 38, and 48 h after surgery are shown in Figure 1. ~~In comparison to 70% PH group, all parameters in 90% PH group showed significant worsening after 30 h.~~ All parameters in the sham group naturally maintained within the normal range at all time points. AST level at 22, 30, 38, 48 h and ALT level at 30, 38, 48 h after surgery were significantly higher in the 90% PH group than in the 70% PH group ($p=0.015$, $p=0.013$, $p=0.031$, $p=0.017$, and $p=0.005$, $p<0.001$, $p<0.001$, respectively). In terms of TBIL and PT-INR which are currently used to diagnose PHLF in a clinical setting, TBIL in the 90% PH group constantly increased from 14 h after surgery until the time of sacrifice and showed higher level at 38 and 48 h compared to 70% PH group ($p=0.001$, and $p=0.002$, respectively). PT-INR from 14 h after surgery was significantly higher in the 90% PH group than in the 70% PH group ($p=0.004$, $p=0.002$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively).

(Discussion)

The adequacy of experimental models used in this study is well supported by *Figure 1*~~,~~. ~~which shows dramatic increase in bilirubin levels and PT-INR in the 90% PH group or their returning trends toward baseline level in the 70% PH group over time.~~ AST and ALT are present in the hepatocytes, and these enzymes leak into the serum when hepatocytes are damaged in various conditions (31). Therefore, higher AST and ALT levels in the 90% PH group indicated more ongoing hepatocyte injury compared to the 70% PH group. TBIL and PT-INR reflecting excretory and synthetic function of the liver (2) dramatically increased in the 90% PH group or showed returning trends toward baseline level in the 70% PH group over time. These four biochemical parameters are widely used to assess the functional status of the liver in a clinical setting.

Comment 2. The second section of results should be included together with the third one, as you do not provide any Figure related to this point but only supplementary material. I strongly recommend to change the significant representation, avoiding the use of discontinued line squares and using *p<0.05 to make lecture much clearer.

Reply 2: Thank you very much for your suggestion. As your recommendation, we combined the second and third sections in the result with a single subtitle. Furthermore, we modified the method of showing statistical significance in *Figure 4 and 6* to bring more evident meanings by using the asterisk instead of a different type of squares.

(Figure legends)

Figure 4 Plots of the fold changes (vs. PO) in lipid classes showing significant changes ~~(> 2-fold and p < 0.05, marked with dotted box)~~ between the 70% PH and 90% PH groups (A) at 30 h and (B) at 48 h after the operation. Dots indicate the means, and whiskers indicate the standard error of the means. *, > 2-fold and $p < 0.05$ vs. 70% PH group in the same time point.

Figure 6 Fold changes (vs. PO) of 14 among 184 quantified lipid species showing (A) significant differences ~~(> 2-fold, p < 0.01, marked with dotted rectangles)~~ between the 70% PH and 90% PH groups at both 30 and 48 h after the operation, and (B) significant differences ~~(p < 0.05, marked with gray rectangles)~~ between the two resection groups either from 14 or 30 h and maintaining the significance thereafter, which are not included in (A). The percentages in parentheses next to the name of each lipid species represent the abundance proportion in each lipid class. PC plasmalogens and TGs exhibited an opposite trend between the two resection groups. Lipid levels of four PC plasmalogens increased at 14 h in both 70% PH and 90% PH groups. However, those in the 70% PH group returned to the level similar to those at PO after 30 h while those in the 90% PH group continued to increase or maintained at higher level compared to the 70% PH group. On the other hand, eight TG species continuously decreased

in the 90% PH group while those in the 70% PH group increased abruptly to a larger degree (4~6 folds) at 30 h followed by decreases at 48 h; the levels at 48 h were nonetheless much higher than those observed in the 90% PH group. Dots indicate the means, and whiskers indicate the standard error of the means. *, > 2-fold with $p < 0.01$ and **, $p < 0.05$ vs. 70% PH group in the same time point.

Comment 3. The discussion is well written and provides a summary and interpretation of the obtained results. I would suggest to include a "Moving forward" part, mentioning in which future research might this work become useful.

Reply 3: Thank you for your suggestion. As your important comment, we revised the discussion as follows.

(Discussion)

Rather, ~~further investigation with those candidates in the clinical settings with or without interventions may shed further lights on their potential values.~~ human-derived studies are warranted to establish a model for predicting PHLF in the early postoperative period by combining the recovery and failure lipid markers identified in this study. Eventually, it may pave the way to overcome PHLF by enabling the rapid application of potential treatments and evaluating their effects.

Reviewer B

General Comments: The early diagnostic biomarkers are important since clinical diagnosis of post-hepatectomy liver failure (PHLF) can only be made on or after the 5th postoperative day. Jo et al analyzed plasma lipidome of porcine hepatectomy models to investigated PHLF associated lipids. Characteristic lipidomic signatures of PHLF could be as a tool for early diagnosis or may open new paths to the study of PHLF. Paper looks interesting but I have reservations regarding this manuscript.

Comment 1. PHLF is known to be associated with the remnant liver volume and functional quality of liver parenchyma, but why author use the 70% and 90% partial hepatectomy (PH) groups as study models of PHLF?

Reply 1: Thank you very much for reviewing our manuscript and your important comment. As your comment, underlying liver function and extent of resection are key contributing factors in recovering from liver resection or developing PHLF. The median age of animals in this study was 3 months, which means they had a healthy liver. In terms of the extent of liver resection, 70% PH was tolerable for a healthy liver due to its regenerative capacity. However, previous studies have reported that animals undergoing 90% PH could not survive for more than 2 days because of PHLF. Therefore, we used the 90% PH model to induce PHLF. As your comment, we revised the discussion as follows.

(Discussion)

We employed two PH models that have a different extent of liver resection. The 70% PH model was used as a normal recovery group after massive hepatectomy, considering that 30% of remnant liver volume is accepted to recover with its regenerative capacity (28). On the other hand, the 90% PH model was regarded as a clinically relevant model ~~We used 90% porcine PH model~~ to induce PHLF (29). ~~and 70% PH model for normal recovery group after major hepatectomy. Since Chen et al.~~ Previous studies have reported that all animals in their PHLF model died before 51 h following hepatectomy (30). Therefore, end-point in the present experiment was set as 48 h following hepatectomy.

Comment 2. Although this study will provide an insight into the relation of PHLF with liver function and regeneration, it is an animal model. I suggest that the content of this paper need to discuss the previous work of human-derived plasma on PHLF and metabolomics or lipidomics and to show what new information their work is bringing to the field.

Reply 2: Thank you for your suggestion. Recently, one study reported a serum metabolomic biomarker model for predicting postoperative mortality using a massive hepatectomy porcine model. However, our study is the first report that aimed to investigate the time-dependent changes in the individual lipid levels after extensive hepatectomy in a large animal model. There have been some human-derived studies about lipidomic analysis of non-alcoholic fatty liver disease or non-alcoholic steatohepatitis. These studies focused on changes in lipids in chronic liver disease. However, the subject of our research was lipidomic analysis before and after massive hepatectomy, causing rapid and dramatic damages to the liver. Therefore, it is hard to correlate or discuss with previous studies. In the near future, human-derived studies are warranted to validate lipid markers identified in this study. We revised the discussion as follows.

(Discussion)

An important hurdle in investigating PHLF in a clinical setting is difficulties in obtaining well-controlled investigational materials from patients in a reasonable time-frame. In contrast, various porcine models of hepatectomy for the study of liver failure have been developed (24-26), which can provide reasonable alternative research materials for this grave condition. Although a recent study reported a serum metabolic biomarker to predict mortality risk associated with extensive hepatectomy (27), lipidomic analysis to evaluate the time-dependent changes in the individual lipid levels using a PHLF model has not yet been investigated.

Comment 3. Clinical diagnosis of PHLF is used the serum bilirubin and prothrombin time. Authors determined the values of ALT, AST, bilirubin and prothrombin time in different time points. The correlations between these selected metabolites and these clinical parameters might be present. I suggest that authors can show the correlation between these significantly changed metabolites with clinical diagnostic markers and might be utilized to predict the early stage of PHLF.

Reply 3: Thank you for your important comment. Serum TBIL and PT-INR on postoperative day 5 are the most widely used biochemical parameters to diagnose PHLF (1). In other words, it implies that a high level of TBIL and PT-INR in earlier postoperative period than 5 days cannot accurately predict the development of PHLF. They only had a 59% sensitivity to predict postoperative mortality (2). Therefore, as we described in the final part of the discussion, correlation of certain lipid species with biochemical parameters in the early postoperative period does not necessarily imply higher potential as a biomarker.

(Discussion)

Considering both that serum TBIL and PT-INR are the most widely used biochemical parameters reflecting liver function and that they cannot accurately predict PHLF by postoperative day 5, significant correlation of certain lipid species with those in the early postoperative period does not necessarily imply higher potential as a biomarker. Rather, ~~further investigation with those candidates in the clinical settings with or without interventions may shed further lights on their potential values.~~ human-derived studies are warranted to establish a model for predicting PHLF in the early postoperative period by combining the recovery and failure lipid markers identified in this study. Eventually, it may pave the way to overcome PHLF by enabling the rapid application of potential treatments and evaluating their effects.

Comment 4. The liver plays important roles in lipid metabolism. Cholesterol and cholesterol related metabolites such as bile acids are also important lipids that can be synthesized by liver. Why didn't show these lipids in this study? Are significant changes of these metabolites in PHLF or not?

Reply 4: Thank you for your comment. Cholesterols are not readily analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) due to their low ionization efficiency. It is usually analyzed by gas chromatography/mass spectrometry (GC/MS) after derivatization reaction, which was not intended in this study. The present study was aimed to investigate lipid categories of glycerophospholipids, glycerolipids, and sphingolipids.

Reference of response to review comments

1. Rahbari NN, Garden OJ, Padbury R, et al. Posthepatectomy liver failure: a definition and grading by the International Study Group of Liver Surgery (ISGLS). Surgery 2011;149:713-24.
2. Balzan S, Belghiti J, Farges O, et al. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. Ann Surg 2005;242:824-9.