

Dear Editor-in-Chief,

Thank you for your letter and the Reviewers' comments on our manuscript entitled "Morbidity after Curative Resection for Hilar Cholangiocarcinoma Adversely Impacts Oncological Prognosis" (Manuscript ID 71260). These comments are valuable to help us to revise and improve our study. We have studied the comments carefully and made amendments which we hope could meet with the Reviewers' and your approval.

I enclose herein a revised manuscript which includes the details of our responses to the Reviewers' comments. The revised portions are underlined in red. Please find enclosed our point-by-point responses to the Reviewers' comments/questions.

Reply to comments and suggestions raised by Reviewer #1:

Reviewer: This is a well-organized paper, but I have some suggestions.

1. Comment: Please describe figure 1 explanation in the main text.

Response: Thank you for the comment. We have described the explanation of Figure 1 in the results section.

"With predetermined inclusion criteria, 239 patients were enrolled in our study (Figure 1)."

2. Comment: Please clarify minor and major morbidity rate in the text and Table 1.

Response: Thank you for the comment. We have reworded the minor and major morbidity rates in the results and discussion sections. The minor and major morbidity

rates in Table 1 were correct.

“Of these patients, 146 (61.1%) experienced morbidity within 30 days of surgery, with minor morbidity occurring in 78 (32.6%) and major morbidity in 68 (28.5%) patients.”

“In this study, postoperative morbidity occurred in 146 (61.1%) patients, of which 68 (28.5%) experienced major morbidity.”

3. Comment: The authors described preoperative CA 19-9 were more common in patients with morbidity, but there is no difference between two groups as shown in Table 2.

Response: Thank you for the comment. We have revised this error in the results section. Preoperative CA 19-9 was not more common in patients with morbidity, as shown in Table 2.

“Notably, obesity, diabetes mellitus, cirrhosis, and intraoperative blood loss > 500 ml were more common in patients with morbidity ($P < 0.05$).”

4. Comment: In this study, patients who received adjuvant chemotherapy were excluded, but I think the role of adjuvant chemotherapy is important for cholangiocarcinoma patient after curative resection. Because adjuvant chemotherapy cannot be administered immediately when complications occur after surgery, so what do you think will affect the prognosis. It would be better put these in the discussion section.

Response: Thank you for the comment. We agree with the reviewer's opinion that adjuvant chemotherapy is very important for the prognosis of HCCA patients after radical surgery. However, as the reviewer said, patients with postoperative morbidities cannot receive adjuvant treatment immediately. To better assess the impact of postoperative morbidity on prognosis, we excluded patients receiving adjuvant chemotherapy and radiotherapy. At the same time, we have added the content in the last paragraph of the discussion.

“In addition, in this study, patients who received adjuvant chemotherapy and radiotherapy were excluded. Some previous studies have demonstrated a benefit of prognosis for patients following surgery who received postoperative adjuvant therapy^[1, 2]. However, adjuvant therapy cannot be administered immediately when morbidities occur after surgery. As a result, we believed it was better to exclude patients who received adjuvant therapy to more accurately reflect the impact of postoperative complications on prognosis.”

[1] Grendar J, Grendarova P, Sinha R, Dixon E. Neoadjuvant therapy for downstaging of locally advanced hilar cholangiocarcinoma: a systematic review. *HPB (Oxford)* 2014; 16: 297-303 [PMID: 23981000 DOI: 10.1111/hpb.12150]

[2] Gerhards MF, van Gulik TM, González González D, Rauws EA, Gouma DJ. Results of postoperative radiotherapy for resectable hilar cholangiocarcinoma. *World J Surg* 2003; 27: 173-179 [PMID: 12616432 DOI: 10.1007/s00268-002-6434-1]

Reply to comments and suggestions raised by Reviewer #2:

Reviewer: Although postoperative morbidity after curative resection for hilar cholangiocarcinoma (HCCA) is common, there have been few reports whether postoperative morbidity has an impact on oncological prognosis. Therefore, the

authors aimed to evaluate the influence of postoperative morbidity on tumor recurrence and mortality after curative resection for HCCA. Postoperative morbidity (especially major morbidity) is revealed to be an independent risk factor for unfavorable prognosis. This is an interesting study; however, I have the following comments and questions.

1. Comment: In the abstract, “postoperative morbidity” and “major morbidity” should be defined objectively, using such as Clavian Dindo classification.

Response: Thank you for the comment. “Postoperative morbidity” and “major morbidity” were defined in the abstract section using the Clavian-Dindo classification.

“Postoperative morbidities were divided into five grades based on the Clavian-Dindo classification, and major morbidities were defined as Clavian-Dindo \geq 3.”

2. Comment: In the abstract, the following sentence, “the median OS and RFS of patients with morbidity were less favorable”, shows weak meaning, because the difference between the two groups was statistically significant.

Response: Thank you for the comment. We have reworded this sentence as suggested by the reviewer.

“the median OS and RFS of patients with morbidity were less favorable.”

“Postoperative morbidity was associated with decreased OS and RFS.”

3. Comment: In core tip, you mention, “It was the first study to investigate the

oncological prognosis of hilar cholangiocarcinoma with postoperative morbidity”, however, the previous study like yours had been already reported, as “.Post-operative morbidity results in decreased long-term survival after resection for hilar cholangiocarcinoma (HPB 2011, 13, 139–147)“

Response: Thank you for the comment. We have deleted this sentence.

~~**“It was the first study to investigate the oncological prognosis of hilar cholangiocarcinoma with postoperative morbidity.”**~~

4. Comment: There is no explanation on Figure 1 in the text, and Figure 1 A and B appearing in the text (survival curves) should be Figure 2A and B.

Response: Thank you for the comment. We have described the explanation of Figure 1 in the results section, and we have modified (Figure 1A and B) to (Figure 2A and B) in the results section.

~~**“With predetermined inclusion criteria, 239 patients were enrolled in our study (Figure 1).”**~~

~~**“(Figure 1 A and B)”**~~

~~**“(Figure 2 A and B)”**~~

5. Comment: In the text, you describe, “with minor morbidity occurring in 91 (38.1%) and major morbidity in 55 (23.0%) of patients”, however, In Table 1, minor morbidity in 78 patients (32.6%) and 68 patients (28.5%). Which is correct?

Response: Thank you for the comment. The data in Table 1 were correct. We have

revised this error in the results section.

“Of these patients, 146 (61.1%) experienced morbidity within 30 days of surgery, with minor morbidity occurring in 78 (32.6%) and major morbidity in 68 (28.5%) patients.”

6. Comment: Some types of postoperative complications overlap, such as biliary infection, bile leak, and cholangitis. For example, biliary infections, bile leaks, cholangitis, etc. are overlapping, as are SSIs, abdominal infections, and wound dehiscence. There should be clearer definitions on this matter.

Response: Thank you for the comment. We classified bile leak, biliary infections and cholangitis as bile leak and classified SSI and abdominal infections as SSI. We redefined them in the methods section and modified the data in Table 2. In addition, we have described the incidence of postoperative morbidity in the results section based on the updated data in Table 2.

“Bile leakage was defined as a drain bilirubin concentration of more than three times higher than that of serum.”

“Surgical site infection was diagnosed based on the Prevention of the National Nosocomial Infections Surveillance and Centers for Disease Control.”

“The top four causes of morbidity were surgical site infection (36/239, 15.1%), bile leak (32/239, 13.4%), PHLF (27/239, 11.3%) and pleural effusion (24/239, 10.0%).”

7. Comment: In the patient characteristics, the following sentence, “Postoperative morbidity was experienced by 146 (61.1%) of the total 239 patients.”, is not required because it has been already mentioned in peroperative outcomes. Instead, “Table 2 shows comparisons of patients’ clinicopathologic and operative variables between patients with and without postoperative morbidity.”, should be mentioned.

Response: Thank you for the comment. We have deleted the following sentence: “Postoperative morbidity was experienced by 146 (61.1%) of the 239 patients” as requested by the reviewer. We have added the following sentence: “Table 2 shows the comparisons of patients’ clinicopathologic and operative variables between those with and without postoperative morbidity.”

~~“**Postoperative morbidity was experienced by 146 (61.1%) of the total 239 patients.**”~~

~~“**Table 2 shows the comparisons of patients’ clinicopathologic and operative variables between those with and without postoperative morbidity.**”~~

8. Comment: In the text, you describe like “obesity, diabetes mellitus, cirrhosis, preoperative CA19-9 > 150 U/L, and intraoperative blood loss > 500 ml were more common in patients with morbidity (P <0.05)”, however, In Table 2, preoperative CA19-9 doesn’t show any significant difference.

Response: Thank you for the comment. We have revised this error in the results section. Preoperative CA 19-9 was not more common in patients with morbidity, as shown in Table 2.

“Notably, obesity, diabetes mellitus, cirrhosis, and intraoperative blood loss > 500 ml were more common in patients with morbidity ($P < 0.05$).”

9. Comment: You describe, “based on the severity of postoperative morbidity, major morbidity was associated with both lower OS”, however, there is no exact survival data. If you add the data of OS and RFS according to major and minor morbidity in Figure 2, it will be more impressive. For example, Figures 2 A and B should have three survival curves of the patients without postoperative morbidity, those with minor morbidity and those with major morbidity. Table 4 already shows OS and RFS data according to with and without postoperative morbidity. Therefore, Figure 2 should have three survival curves.

Response: Thank you for the comment. We deeply agree with the reviewers’ opinions that Figure 2 should have three survival curves. However, in my opinion, OS and RFS curve comparisons between patients with and without postoperative morbidity are also required. As a result, we added Figure 3 A and B to show the OS and RFS curve comparisons among patients without postoperative morbidity, with minor postoperative morbidity, and with major postoperative morbidity.

“Furthermore, based on the severity of postoperative morbidity, major morbidity was associated with both lower OS and RFS, as shown in Figure 3 A and B (OS: HR: 2.175; 95% CI: 1.470-3.216, $P < 0.001$; RFS: HR: 2.054; 95% CI: 1.400-3.014, $P < 0.001$).”

Reply to comments and suggestions raised by Reviewer #3:

Reviewer: Well-conducted and well-written study on a topic that has not been extensively investigated to date. Some specific comments are shown below.

1. Comment: Please provide IRB and Biostatistics Certificate in English.

Response: Thank you for the comment. We apologize to the reviewer that our hospital can only provide IRB and Biostatistics Certificate in Chinese.

2. Comment: Figure 1 is not properly cited in the text. The authors have confused Figure 1 with Figure 2.

Response: Thank you for the comment. We have described Figure 1 in the results section. We have clarified the content of Figure 1 and Figure 2.

“With predetermined inclusion criteria, 239 patients were enrolled in our study (Figure 1).”

“The median OS and RFS were significantly lower in the patients with postoperative morbidity, as shown in Figure 2 A and B (OS: 18.0 vs. 31.0 months, $P = 0.003$; RFS: 16.0 vs. 26.0 months, $P = 0.002$).”

3. Comment: There seems to be a problem with the data. How is the number at risk higher for RFS compared to OS in Figure 2 at 60 months? Didn't this last patient die on the first figure panel? Then, why isn't that patient having an event in the second figure panel? Please review all your survival data.

Response: Thank you for the comment. We reviewed all of the survival data and

found that the recurrence status of two patients was entered incorrectly. We apologize for this error, and we have reanalyzed all of the survival data involving RFS. We have revised Tables 4, 6 and Figure 2. We have revised the corresponding values in the abstract and results section.

4. Comment: Were all cases performed open?

Response: Thank you for the comment. All of the patients performed open surgery, and we have mentioned this in the Results section.

“All patients performed open surgery.”

5. Comment: How many cases required vascular reconstruction and how many had a Pringle maneuver? Please include in Table 2 and further analyses separately for arterial and venous reconstruction.

Response: Thank you for the comment. We have added the number of hepatic artery reconstructions, portal vein reconstructions, and Pringle maneuvers to Table 2. In addition, we have further analyzed the data in Table 2.

6. Comment: How many patients underwent lymph node dissection and how many lymph nodes were resected? Please include in Table 2 and further analyses.

Response: Thank you for the comment. All of the patients underwent lymph node dissection, and we have mentioned this in the Methods. When positive lymph nodes are not found, evaluation of less than four lymph nodes can cause understaging^[1].

Therefore, we have added the proportion of lymph node examinations > 4 in Table 2, and we have further analyzed the data.

“Regardless of preoperative computed tomography (CT), magnetic resonance imaging (MRI) or suspicion of lymph node metastasis, all patients underwent locoregional lymphadenectomy.”

7. Comment: How many patients underwent lymph node dissection and how many lymph nodes were resected? Please include in Table 2 and further analyses.

Response: Thank you for the comment. All of the patients underwent lymph node dissection, and we have mentioned this in the Methods. We have added the number of resected lymph nodes to Table 2, and we have further analyzed the data.

8. Comment: I agree with the authors' Discussion that propensity score matching is not required.

Response: Thank you for the comment.

Reply to comments and suggestions raised by Reviewer #4:

Reviewer: The authors present a work entitled "Morbidity after curative resection for hilar cholangiocarcinoma adversely impacts oncological prognosis". In their work, they highlight the incidence of postoperative morbidity (major and minor) following HCCA resection and identify risk factors for the development of postoperative morbidity. They also show that postoperative morbidity adversely affects the

oncologic prognosis from the perspective of recurrence-free and overall survival.

1. Comment: First, please be sure to re-read for grammatical accuracy with respect to the English language. There are small grammatical errors throughout that need to be addressed.

Response: Thank you for the comment. We have sent this article to AJE for revision according to the request of the journal. Some small grammatical errors have been addressed.

2. Comment: Second, the statistical analyses are well done, and the conclusions from these data are well-made. HCCA resection is a very morbid procedure, and the authors found that pre-existing conditions such as cirrhosis, diabetes, and obesity increase the risk of morbidity. The authors correctly note that preoperative medical optimization may lessen this risk, but what about the increased operative blood loss as an independent risk? Perhaps one solution could be the use of a different type of electrocautery - either bipolar as opposed to traditional monopolar? Or plasma, which has been shown to have some anticancer efficacy? Or water sealed bipolar? I think this merits exploration.

Response: Thank you for the comment. We have revised the discussion on the risk factors for intraoperative blood loss.

~~“Considering the surgery itself, greater intraoperative bleeding is an important reason for intraoperative blood transfusion which has been found to be predictive of postoperative morbidity”~~

“For patients with poor liver function and coagulation dysfunction, intraoperative infusion of plasma or cryoprecipitate may help to reduce intraoperative bleeding.”

“Moreover, the vast majority of intraoperative bleeding occurs during liver resection. With new medical advances, many kinds of instruments can be used for liver resection: ultrasonic knife, electrocautery (bipolar, monopolar, or water sealed bipolar), and radiofrequency-assisted liver resection. However, which can better prevent intraoperative bleeding may be related to the patient's liver condition and the operator's habits, and it is worthy of further study.”

3. Comment: Third, I would caution the authors on drawing conclusions on the influence of various non-biological factors on RFS. Tumor recurrence is due to inherently biological characteristics. The authors are correct in saying that tumor histological properties such as microscopic invasion and degree of differentiation can increase the risk of a poor RFS, but I doubt age and ASA score (non-tumor intrinsic properties) have a reliable association with recurrence. These variables should likely have been defined a priori. Does the final Cox model change when you omit clinical characteristics and use only pathological tumor traits for RFS?

Response: Thank you for the comment. In this study, variables found significant at $P < .100$ in univariable analyses were entered into multivariable Cox regression analyses. The P values of age and ASA score in the univariable analyses associated with RFS were 0.330 and 0.457, respectively. Therefore, age and ASA score were not entered

into the final Cox model in this study.

4. Comment: Last, I think the authors adequately detail the current literature with respect to morbidity and HCCA resection. As mentioned, this is a morbid procedure, and certainly larger studies may be necessary to help establish new treatment algorithms for these patients, which was a limitation mentioned.

Response: Thank you for the comment. In the future, we plan to establish a multicenter large sample database to obtain more affirmative conclusions.