

Dear Editor:

We would like to resubmit the revised manuscript (The prognostic value of lymph nodes count on survival of patients with distal cholangiocarcinomas, Manuscript NO.: 37585) for consideration by World Journal of Gastroenterology. We would like to thank the reviewers for thoroughly reviewing our manuscript and making many thoughtful comments. We have added significant new data, described in detail below, and revised the manuscript to address reviewers' comments. In this revised version, changes to our manuscript were all highlighted within the document by using red colored text and here are our point-by-point responses:

Reviewer #1 (00182423): Thank you for your nice comments on our article. According to your suggestions, we have supplemented several data here and corrected several mistakes in our previous draft. The detailed point-by-point responses for comments are listed below.

Comment 1: It was not easy to understand this manuscript because of complex statistical formulas.

Answer: Thanks for your suggestion. It is very sorry for the complex statistical formulas. To make the statistical method of the present study easy to understand, we illustrated the process of the analysis in detail here. Our study follow the following analysis steps: a) the retrieved LNs counts were transformed from continuous variables to categorical variables, and the cutoff was defined by the X-tile program. The X-tile program identified the cut-off with the minimum P values from log-rank χ^2 statistics for the categorical retrieved LNs counts in terms of survival; b) the OS and CSS were compared between the different categories of retrieved LNs counts by the means of Kaplan-Meier method and Cox regression analysis; c) we performed stratified analyses by the clinical factors that were evaluated to be independently associated with survival in the Cox regression analysis, among patients within the different LNs groups. The above three steps analysis were performed in all, N0 and N1 patients respectively. Actually, the X-tile program from Yale University is a useful and popular tool for outcome-based cut-point optimization. That has been widely used in many studies. And the use of Kaplan-Meier analysis and Cox regression analysis in survival analysis are very common. The statistical method in stratified analyses were just the Kaplan-Meier analysis and Cox regression analysis. Therefore the statistical methods in the present study are actually usual and common, not that complex. Additionally, we added more details and references in the statistical section of the manuscript, but we cannot add too much content here to avoid to make the study seem verbose. Please check that at page 7, line 5-8 and line 11-12.

Comment 2: The authors insisted that retrieved LN counts more than 9 led to a worse prognosis than retrieved LN counts 4-9 in patients with N0 distal bile duct cancer. It was verified by using complex statistical formulas. However, even the authors could hardly explain why. I hope the authors be able to clearly explain why before this manuscript is published.

Answer: Thanks for your reminding. We have talked about the reason why retrieved more than 9 LNs led to a worse prognosis than retrieved 4-9 LNs in patients with N0 distal cholangiocarcinoma in the discussion section. Four hypotheses were proposed to explain why retrieved too many LNs led to a worse prognosis. Please check that at page 10, line 25-30 and page 11, line 1-8. Meanwhile, we also talked about why retrieved less that 4 LNs led to a worse prognosis than retrieved 4-9 LNs. Another four hypotheses were proposed to explain why

retrieved too less LNs led to a worse prognosis. Please check that at page 10, line 2-9. Therefore patients with 4-9 retrieved LNs are of without shortcomings of the retrieving too many and less LNs the same time, and bear a better prognosis. But we could not figure out, which one or several of the above hypotheses played the major rule, just rely on the present retrospective study that based on a population-based database. Therefore we hope the future study could improve upon the weaker areas of present study to find out the clear reason why patients with 4-9 retrieved LNs bear a better prognosis than others.

Comment 3: More specifically in Table 2, OS and CSS were the best when the number of retrieved nodes was 7 in N0 patient group. However, in the case of 5 retrieved LN (belong to the best tier according to the 3 tier system in this manuscript), OS and CSS were quite low, 30% and 37.5%, respectively. These figures were worse than the cases of retrieved LN number 1, 3, 11, 13, 21 and 25. And the distribution of OS and CSS in the case of 11 ~ 25 did not show a constant trend. Although the results showed a statistical significance, it should be judged more carefully. It is possible confounding factors were behind the scene.

Answer: Thanks for your suggestions. A) The reviewer found out that patients with 5 retrieved LNs had a quite low OS and CSS in the Table 2. The reasons for the above problem are as follows: a) the results in the Table 2 were only based on univariate survival analysis without adjusting for other clinical factors, and we found that 8 of the 11 patients with 5 retrieved LNs were more than 70 years old and nearly half the patients (n=5) were with stage III tumors; b) the number of patients with 5 retrieved LNs was low, therefore the selection bias might lead to this result; c) the aim of the present study was to find out a category of retrieved LNs counts, not a single number of retrieved LNs, with the best prognosis.

B) The reviewer also found out that the distribution of OS and CSS in the case of 11 to 25 did not show a constant trend in the Table 2. To evaluate if there was any heterogeneity in terms of prognosis in patients with 11 to 25 retrieved LNs, we performed survival analysis after divided the patients with 11-25 retrieved LNs into two groups (the cutoff number for grouping was 15 which was determined by the X-tile program). And we found out that patients with 4-9 retrieved LNs still had a better prognosis than other patients (unadjusted $p=0.019$, Figure 1, adjusted $p=0.008$). Patients with more than 15 retrieved LNs had a better prognosis than patients with 11-15 retrieved LNs (this made the distribution of OS and CSS in the case of 11 to 25 do not show a constant trend in the Table 2), but the difference was not significantly ($p=0.135$, Figure 2). Therefore there was no heterogeneity in terms of prognosis in patients with 11 to 25 retrieved LNs, and the reasons for why patients with more than 15 retrieved LNs had a moderately better prognosis than patients with 11-15 retrieved LNs might be that the selection bias resulted from small sample size, retrieving more LNs to remove more micrometastases in the negative LNs, the influence from other confounding factors or some other reasons. However this problem did not have any influence on the results of the present study (patients with 4 to 9 retrieved LNs still had the best prognosis) and we hope this problem could be studied in the further research.

C) The reviewer commented that our results might be influenced by other confounding factors that behind the scene and should be judged more carefully. We agree with the comments of reviewer. Several factors such as the lymph nodes distant from the lesion might have influence on our results, but we could not add those factors into our analysis due to the limitation of SEER database. We have discussed this problem in the limitation section, please check that at page 11,

line 17-19. The same time, we emphasized that the clear mechanism for our results was still unclear and the results should be validated in the future studies, in the conclusion section. We hope that you will be satisfied with the revision.

Figure 1

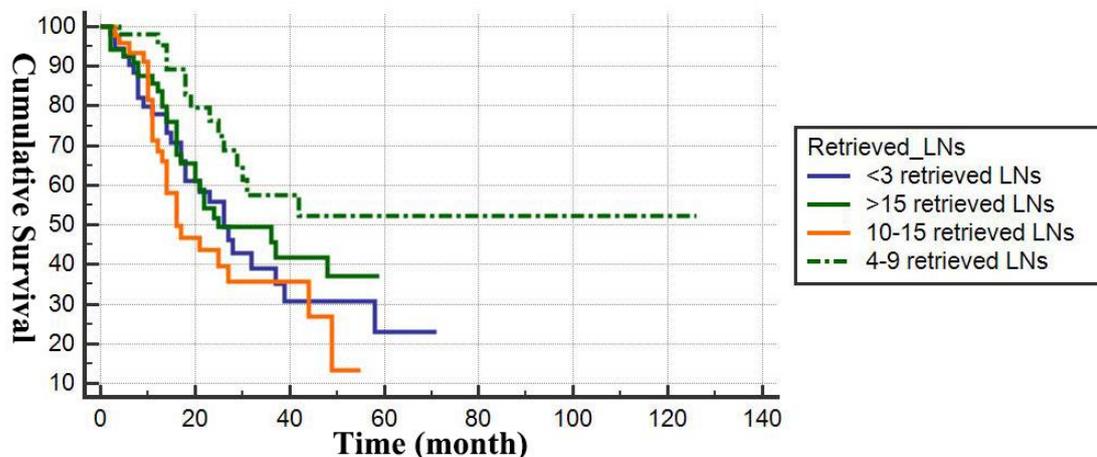
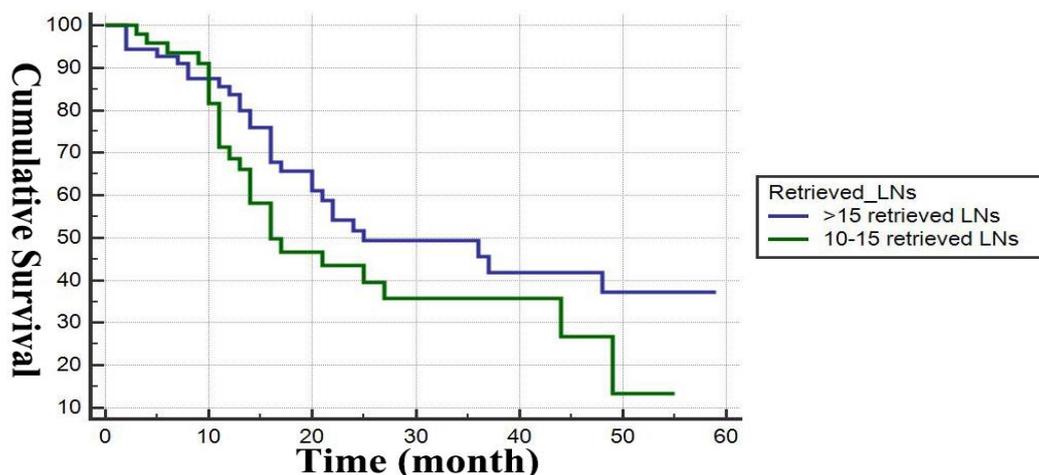


Figure 2



Reviewer #2 (03253499): Thank you for nice comments on our article. Your comments are all of great importance to our article. All of these comments have contributed a lot to improve the quality of our article. Based on your comments, we attached a point-by-point responses to you.

Comment 1: The aim of this retrospective study on 449 patients with distal cholangiocarcinoma was to investigate the prognostic impact of lymph nodes (LNs) metastasis and to determine the optimal retrieved LNs cut-off number. To do this the authors have divided patients with a different number of retrieved LNs into three groups. In the results, this study denoted that retrieving more than 9 LNs did not indicate a better prognosis in patients with node-negative distal cholangiocarcinoma. An increase in terms of all-cause mortality risk and cancer cause-specific mortality risk was observed compared with retrieving 4 to 9 LNs. Patients with distal cholangiocarcinoma, retrieving too many LNs did not obtain better outcomes. This paper is well conducted and focused on an interesting topic because it is clear that lymph node status is a strong predictor for the prognosis of patients with distal cholangiocarcinoma, but the number of LNs

should be retrieved is still under debate. The result of this study was contrary to the previous data that a better prognosis was always associated with higher retrieved LN counts. For this reason this paper could stimulate further area of research on this topic.

Answer: Thanks for your comments. We hope our study could stimulate further area of research on this topic and we also hope the future study could improve upon the weaker areas of present study.

Reviewer #3 (02441405): Thank you for your nice comments on our article. According to your suggestions, we have supplemented several data here and corrected several mistakes in our previous draft. We have made extensive revisions to our previous draft. The detailed point-by-point responses are listed below.

Comment 1: This manuscript describes an interesting finding that a medium number (4-9) of lymph nodes dissected predict better overall and cancer specific survival of patients with node-negative distal cholangiocarcinoma than other groups. It may impact us on how extensive lymph node dissection should be. Several questions and comments. The number of lymph nodes retrieved may depend upon the type of surgical procedures, i.e. open location resection, Whipple's procedure, and laparoscopy resection, and also rely on lymph node dissection skill in each individual institution (grossing by resident vs. practicing pathologists or pathologist assistant). The lymph nodes distant from the lesion (for example, nodes from Whipple's procedure) may not have the same predicting value as these from local or limited resection specimen. It will be helpful if authors can consider above parameters (i.e. types of procedure and setting of surgery such as tertiary or community hospitals) into analysis.

Answer: Thanks for your suggestions. We quite agree with the comments of reviewer. The number of lymph nodes retrieved may depend upon the type of surgical procedures and setting of surgery (tertiary or community hospitals). However, the detailed operation methods for patients with distal cholangiocarcinomas were unknown because of the limitation of the SEER, i.e., we could not know how many patients underwent pancreatoduodenectomy or segmental bile duct resection. But we could figure out which part of patients underwent extensive surgery or local excision, and this parameter has been added into our analysis, please check that in the Table 3 and 4. And the SEER database only provided the information of the region where the patients from, the classes of hospital were unknown. Therefore we discussed the above two parameters in the limitations section of the present study. Please check that at page 11, line 17-19 and line 21-25. And we also mentioned this in the conclusion section, we hope the future studies could consider the detailed type of surgical procedures and setting of surgery into their analysis to evaluate our results.

Comment 2: In line 20 and 21, the statement "most patients underwent extensive surgery and chemotherapy" is ambiguous. Authors need to clarify whether "chemotherapy" is neoadjuvant therapy or post-operative therapy. In the context of the manuscript, patients included in the study should not undergo neoadjuvant therapy.

Answer: Thanks for your reminding. We have modified the chemotherapy to post-operative chemotherapy and radiotherapy to adjuvant radiotherapy in this sentence. Please check that at page 7, line 23-25. The patients that received neoadjuvant therapy have been excluded from the

present study. We hope that you will be satisfied with the revision.

Comment 3: The manuscript provides information that the predictive value of the number of lymph nodes is independent from pathological T stage. Patients with node-negative carcinoma can be classified as clinical stage I, II, IIB, or IIB. Have authors compared the survival among the groups of patients with 4-9 lymph nodes and others based upon clinical stage?

Answer: Thanks for your suggestion. The present study was based on the data from the SEER database, however, only pathological stage information was provided for patients with resectable distal cholangiocarcinomas. Therefore we could not compared the survival among the groups of patients with 4-9 lymph nodes and others based upon clinical stage due to the limitation of SEER database. And we listed this as one of the limitations of our study, please check that at page 11, line 26-29.

Comment 4: Does the tumor grade or differentiation correlate with the number of lymph nodes dissected? Is there difference of tumor grade or differentiation between the groups of patients with 4-9 lymph nodes and other groups?

Answer: Thanks for your suggestions. We made a table below to show the relationship between the tumor grade and the number of lymph nodes dissected. Subsequently, we performed a logistic regression analysis to evaluate if the tumor grade correlated with the number of lymph nodes dissected, and found that the grade of tumor was not significantly correlated with the number of lymph nodes dissected ($p=0.623$). We performed a Chi square test to evaluate if there was any difference of tumor grade between patients with different number of lymph nodes dissected, and found that there was no significantly difference of tumor grade between the groups of patients with 4-9 lymph nodes and other groups ($\chi^2=4.54$, $p=0.604$). We hope that you will be satisfied with the revision.

Grade	Patients with 1-3 retrieved	Patients with 4-9 retrieved	Patients with > 9 retrieved
	LN _s (n=54)	LN _s (n=52)	LN _s (n=117)
Well differentiated	10	7	19
Moderately differentiated	24	26	49
Poorly or Undifferentiated	13	16	41
Unknown	7	3	8

According to the reviewer's comments, we have revised the manuscript extensively. If there are any other modifications we could make, we would like very much to modify them and we really appreciate your help. World Journal of Gastroenterology is a journal of great popularity and prestige. We hope that our manuscript could be considered for publication in your journal. Thank you very much for your help and your consideration of our manuscript.

Yours sincerely,
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