Peer Review File

Article Information: https://dx.doi.org/10.21037/jgo-23-950

Reviewer A

Comment 1: In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply 1: We deeply appreciate your valuable insights. In the Introduction, we referenced two articles to illustrate the existence of distinct prognostic subgroups among early intrahepatic cholangiocarcinoma (ICC) patients, stating, "Due to the varying prognoses within different subgroups of early ICC patients." This highlights the limitations of previous studies, stating, "despite the existing research on PNI in this population, the impact of PNI on the prognosis of early ICC patients still requires further exploration." Subsequently, we introduced the central focus of this study, stating, "We sought to elucidate the predictive influence of PNI in early-stage ICC and assess the value added for the T category of the eighth edition of the AJCC."

Changes in the text: we have modified our text as advised (see Page 4, line 17 to 23).

Comment 2: What is the efficacy of adjuvant therapy in ICC patients with PNI? Please investigate the potential relationship between PNI and postoperative chemotherapy.

Reply 2: We greatly appreciate the insights you provided. Regrettably, we lack the corresponding cohorts, and all early-stage intrahepatic cholangiocarcinoma (ICC) patients included in this study did not undergo postoperative adjuvant therapy. Additionally, as per the current state of pathology, there is no ideal predictive method for perineural invasion (PNI), making it challenging to consider the relationship between preoperative adjuvant therapy and PNI. Therefore, we conducted a literature search and cited relevant studies in the manuscript, stating, "While our study focuses on early-stage ICC patients without further postoperative adjuvant therapy, existing literature has reported that PNI-positive ICC patients exhibit insensitivity to postoperative chemotherapy (10). However, following combined immunotherapy, the prognosis of PNI-positive postoperative patients is superior to that of negative patients (18,19)." Your question has provided valuable insights, and we will explore potential methods to predict PNI preoperatively.

Changes in the text: we added some references (see Page 6, line 37 to 41).

Comment 3: What are the clinical implications and molecular features of ICC with PNI? It is recommended to add relevant content.

Reply 3: Thank you for your suggestions; this is also a part that our manuscript lacked. We carefully reviewed recently published literature and observed that elevated levels of certain molecules are often associated with a poorer prognosis in PNI-positive patients, stating, “In PNI-positive ICC patients, molecules associated with a poorer prognosis for ICC, such as Y1R, A1AT, GPX4, and Kras mutations, exhibit elevated levels (33-36)“.

Changes in the text: we added some references (see Page 7, line 11 to 13).

Comment 4: What is the relationship between PNI, clinicopathological characteristics, and long-term survival in the overall cohort and the subset of patients with early-stage ICC? It is

recommended to add relevant content.

Reply 4: Thank you for pointing out the issue; this is indeed an area where we fell short. Therefore, we divided the entire patient cohort into PNI-positive and negative groups and conducted survival analyses. However, for other clinicopathological features we included, such as differentiation, tumor size, tumor number, and degree of liver cirrhosis, we only explored factors with a P-value less than 0.05 in the multivariate analysis. This approach was taken because multivariate analysis can further eliminate the influence of other confounding factors, thereby determining the correlation between predictor variables and response variables. Additionally, we have incorporated relevant citations, "Moreover, PNI is closely associated with the pathological malignancy phenotype of ICC, with patients exhibiting PNI often accompanied by large duct-type and lymph node metastasis (19).".

Changes in the text: we added some data (see Page 6, line 1 to 2 and 7 to 8) and references (see Page 7, line 8 to 10).

Comment 5: This study is a retrospective analysis, which is likely to cause some deviations in the results. It needs to be further confirmed by multi-center clinical trials.

Reply 5: This is a very insightful suggestion, and we have acknowledged in the manuscript that this is indeed a limitation of our study, staining, "The study has limitations, including the retrospective design and the use of data from a single Chinese institution, which could result in inherent patient selection bias. Therefore, conducting a multicenter prospective study is imperative to advance and elucidate the outcomes." Although we are currently unable to undertake large-scale multicenter clinical trials, it does not hinder our commitment to continuing this research.

Comment 6: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “IL23R as an indicator of immune infiltration and poor prognosis in intrahepatic cholangiocarcinoma: a bioinformatics analysis, PMID:37969393”. It is recommended to quote the article.

Reply 6: Thank you for your suggestion. This is a very interesting article that provides important insights into the treatment decisions for ICC patients. Therefore, we have cited this literature in our manuscript.

Changes in the text: we added this paper as the 23rd reference (see Page 6, line 41 to 42).

Comment 7: Figures and tables described in the manuscript are missing, please provide complete information.

Reply 7: Thank you for pointing out the issues. We sincerely apologize for any confusion caused by our oversight. We have corrected the manuscript.

Changes in the text: we corrected the mistake (see Page 6, line 22-23)

Reviewer B

Comment 1: the title needs to indicate the detailed clinical research design of this study such as a retrospective cohort study.

Reply 1: Thank you for your valuable feedback. Indeed, these suggestions will enhance the clarity of our manuscript, making it more appealing to readers interested in similar research.

Changes in the text: we have modified our text as advised (see Page 1, line 2)

Comment 2: the abstract is inadequate. The objective did not indicate the knowledge gap on the prognostic role of PNI. The methods did not describe the inclusion criteria, the assessment of baseline clinical factors and pathological characteristics, follow up procedures, and prognosis outcomes. The results need to briefly summarize the baseline clinical characteristics of the study sample, the correlation coefficients and accurate P values, and HR and accurate P values. The conclusion needs comments for the clinical implications of the findings.

Reply 2: Thank you for your valuable feedback. We have revised the abstract based on your suggestions. In the background section, we have added a description of the limitations of previous studies, emphasizing the clinical significance of our research. In the methods section, we have rephrased the content without providing excessive details to avoid lengthening the abstract, as the detailed description is available in the main methods section. In the results section, we have included precise statistical indicators. In the conclusion, we have emphasized the clinical significance of our findings.

Changes in the text: we have modified our text as advised (see Page 3, line 2 to 24).

Comment 3: the introduction of the main text needs to review what has been known on the prognostic factors in HCC, why PNI is important but clinically understudied, and what the clinical needs to additionally consider T1-2 staging is.

Reply 3: Thank you for your valuable insights. In the Introduction, we have referenced two articles highlighting the presence of distinct prognostic subgroups among early Intrahepatic Cholangiocarcinoma (ICC) patients, stating, "Due to the varying prognoses within different subgroups of early ICC patients," pointing out the limitations in previous research, "despite the existing research on PNI in this population, the impact of PNI on the prognosis of early ICC patients still requires further exploration." Subsequently, we introduce the central focus of our study, stating, "We sought to elucidate the predictive influence of PNI in early-stage ICC and assess the value added for the T category of the eighth edition of the AJCC."

Changes in the text: we have modified our text as advised (see Page 4, line 17 to 23).

Comment 4: the methodology of the main text needs to specify the clinical research design of this study, sample size estimation, and assessment of baseline clinical and pathological factors. In statistics, please describe the details of multiple Cox regression analysis, in particular how the independent prognostic role of PNI was ascertained and the adjusted covariates.

Reply 4: Thank you for your feedback. In response to your suggestions, we have revised our manuscript to emphasize the experimental design and provide a comprehensive evaluation of subsequent clinical and pathological parameters. We have also reorganized and summarized the statistical section. However, we must candidly admit that we did not conduct a sample size estimation; rather, we expanded the sample size based on a literature review of previous ICC studies. This study included a total of 268 early-stage ICC patients, a larger sample size compared to most studies focusing on early ICC. For your reference, please find relevant literature:

"Endoscopic Ultrasound/Fine Needle Aspiration Is Effective for Lymph Node Staging in Patients With Cholangiocarcinoma. Hepatology. 2020 Sep;72(3):940-948. doi: 10.1002/hep.31077. Epub 2020 Jul 9. PMID: 31860935."

"Machine learning radiomics to predict the early recurrence of intrahepatic cholangiocarcinoma after curative resection: A multicentre cohort study. Eur J Nucl Med Mol Imaging. 2023 Jul;50(8):2501-2513. doi: 10.1007/s00259-023-06184-6. Epub 2023 Mar 16. PMID: 36922449."

Changes in the text: we have modified our text as advised (see Page 4, line 27 to 28 and 37 to 43).

Comment 5: Finally, please consider to cite several potentially relevant papers:

1. Cheng Z, Lei Z, Jin X, Zhang Q, Si A, Yang P, Zhou J, Hartmann D, Hüser N, Shen F. Postoperative adjuvant transarterial chemoembolization for intrahepatic cholangiocarcinoma patients with microvascular invasion: a propensity score analysis. J Gastrointest Oncol 2021;12(2):819-830. doi: 10.21037/jgo-20-443.
2. Jiang C, Zhao L, Xin B, Ma G, Wang X, Song S. 18F-FDG PET/CT radiomic analysis for classifying and predicting microvascular invasion in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Quant Imaging Med Surg 2022;12(8):4135-4150. doi: 10.21037/qims-21-1167.
3. Melandro F, Nasto RA, Ginesini M, Balzano E, Bindi ML, Ghinolfi D, Lai Q. A narrative review of intrahepatic cholangiocarcinoma: a surgical curative option. Chin Clin Oncol 2023;12(2):13. doi: 10.21037/cco-22-85

Reply 5: Thank you for your feedback. These three articles are highly insightful and play a crucial role in guiding treatment decisions for ICC patients. Therefore, we have cited these three articles in our manuscript.

Changes in the text: we added these papers from the 20th to 22nd references (see Page 6, line 41 to 42).