Peer Review File

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Reviewer A

Comment 1: In Table 1, the cases for IHCCA and gallbladder cancer are more frequent in female. The proportion between sex is quite different from the other reports in other areas especially in Asian country that IHCCA is more incident in male. The authors should discuss on this point as well as whether this phenomenon can represent the general population or be adaptable for people in the other areas.

Reply 1: Thank you for this comment, as you raise an excellent point. In the current study, female sex was predominant (65%) which would be expected in the case of gallbladder cancer (77% female in our series). Registry data in North America suggests that the incidence of IHCCA is slightly higher in males, although not substantially so; 51.6% of new diagnoses from 1999-2013 in combined US and Canadian registry data were in males (Van Dyke AL Cancer 2019). We feel that the discrepancy between the current study and North American registry data is likely related to sample size variation. You are correct that these results may be more applicable to populations in regions with similar demographic distribution. We have commented on this in the Limitations section of our Discussion:

Page 9, lines 271-276:

"Results from the current series may be less generalizable to populations in other geographic regions, an important consideration given global variations in biliary tract cancer incidence. For example, in the current study the majority of IHCCA cases were in women, while in Asian countries IHCCA has a higher incidence in males."

Comment 2: The IHCCA cases were likely to have poorer prognostic factors, e.g., bigger size of primary tumors, nodal positive, and R1 margin. Whether these factors may affect the recurrence should be consider. The subgroup analysis only in cases of IHCCA classified by these data could be helpful.

Reply 2: Thank you for this comment. It is true that the disease sites had differing rates of high risk features as you note. However, we hoped to address this issue by assessing recurrence free survival between tumor site in a multivariable model to adjust for these differences. We intentionally did not include tumor size as a variable in this model as IHCCA is frequently larger in size due to its mass-forming biology, compared to EHCCA which grows along the bile duct. We feel that by adjusting by T-stage this should address the issue that you raise. Among these risk factors, we only found node positivity to be associated with worse outcome (Table 2).

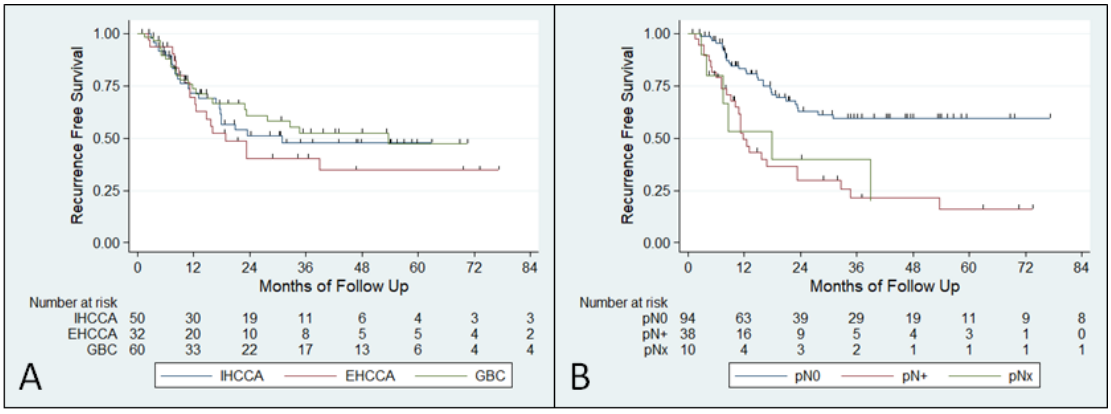
These results are discussed in the manuscript in the Results as follows (Page 6, lines 173-178), but we have edited the text to be more specific as to why we did not adjust for tumor size.

"Multivariable Cox regression was performed to assess for underlying differences in RFS by primary tumor site masked by differences in clinical and pathologic characteristics (Table 2). There remained no significant difference in RFS by tumor site after adjustment for age, sex, pathologic T stage, nodal status, margin status, receipt of systemic chemotherapy, or receipt of chemoradiotherapy. Tumor size was not included in this analysis due to inherent differences in tumor growth patterns by anatomic site (eg, mass-forming versus growth along biliary tracts)."

Comment 3: It was shown that the cases with metastasis were excluded, but those with lymph node involvement (probably considered local invasion) were included for the analysis. As it is difficult to define whether there were already micro-metastases in cases with lymph node positive (presumably more aggressive tumors), the subgroup analysis between node negative and node positive should also be done.

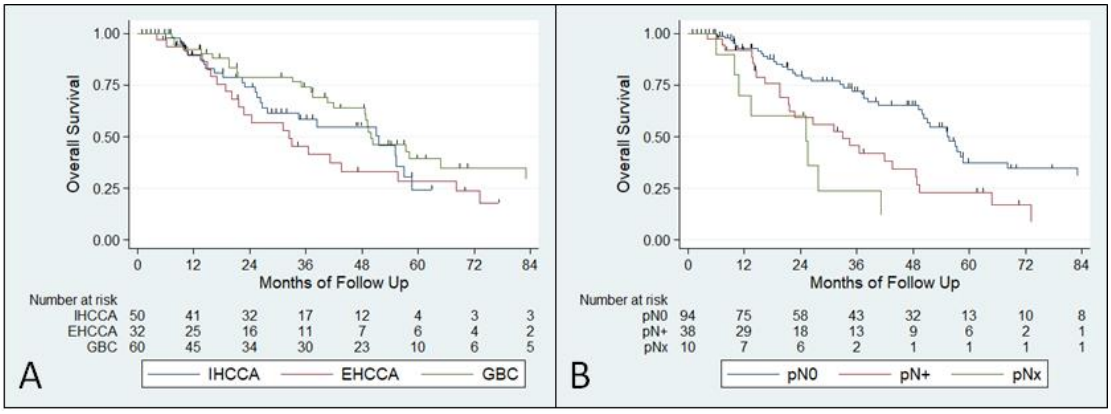
Reply 3: Thank you for this comment. In this study, we focused on surgically resectable cases, which do in our practice include cases with suspicious regional lymph nodes. In the cases of suspicious distant nodes (such as aortocaval nodes), which we would consider distant metastatic disease, these are sampled early in our resections and if found to be positive on intraoperative frozen pathology, complete resection is not performed given high risk of recurrence. These patients are thus not included in this study. We agree that cases with involved regional lymph nodes are likely to have more aggressive disease. In Figure 2 (which is now designated Figure 1B), we found that lymph node positive cases had worse recurrence-free survival (shown

below).



The association between poor RFS and lymph node positivity in Figure 1B remained significant in multivariable Cox regression (Table 2, HR 3.92, p<0.001).

Additionally, as we have revised our manuscript to include analysis of overall survival (OS) at the recommendation of reviewer 2, we explored the association between regional lymph node positivity and OS (Figure 2B below). The association between pN+ and OS remained significant in multivariable Cox regression (HR 2.08, p=0.012).



We have adjusted the Methods section of our manuscript to clarify that our analysis focuses only on regional lymph nodes as patients with involved distant lymph nodes did not undergo resection.

Page 4, lines 126-128:

“As it is institutional practice to intraoperatively sample suspicious distant lymphadenopathy (such

as aortocaval nodes) prior to proceeding with complete resection, node status refers to regional lymph nodes only."

Comment 4: There is a typo in Line 200, "IHHCA".
Reply 4: This had been edited, thank you for your attention.

Comment 5: Please include the ethic approval number and the date of approval in the manuscript.
Reply 5: This has been added, thank you.

Reviewer B

Comment 1: Please add ethnicity in Table 1, if possible, since tumor behaviors might differ.

Reply 1: Thank you for this comment. We have added race/ethnicity to Table 1. Both the IHCCA and EHCCA groups were predominantly white (94% and 97%, respectively), while the GBC cohort was more diverse (15% hispanic, 10% black, 3% Asian). We agree that it is important to report these data so that generalizability to other populations may be considered by readers.

We have added within the Limitations paragraph of the Discussion a comment on this

Page 9, lines 271-276:

"Results from the current series may be less generalizable to populations in other geographic regions, an important consideration given global variations in biliary tract cancer incidence. For example, in the current study the majority of IHCCA cases were in women, while in Asian countries IHCCA has a higher incidence in males. Additionally, the majority of patients were white so racial and/or ethnic influences on tumor biology cannot be well studied in this cohort."

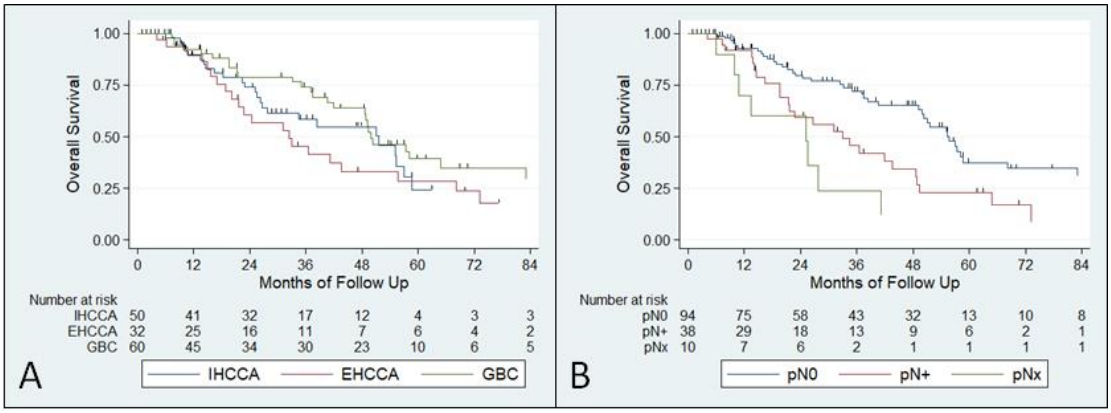
Comment 2: Please add range of follow up period.
Reply 2: This has been added to the Results, Recurrence and Recurrence-Free Survival section

Page 5, line 167-168:

"Median follow up time in this study was 42 months (range 3.3-133 months)."

Comment 3: Overall survival is the most important and robust outcome. Can you add data (and analysis) of survival?

Reply 3: Thank you for your recommendation. We had initially focused our study on recurrence as it may differ between primary tumor site within the biliary tract, but we agree that ultimately overall survival (OS) is the most important outcome we as clinicians assess. We have revised our manuscript to include this outcome (Shown in Figure 2 below; please note Figure 2 was formerly RFS stratified by lymph node status but we have now adjusted this to include that figure within Figure 1). We found no significant difference in OS by primary tumor site, although it should be kept in mind that the patients in this study are all surgically resectable patients; patients with unresectable EHCCA – a common presentation – are not included. Similar to the finding that regional lymph node positivity was found to be the most important determinant of RFS across biliary tract cancers, we found pN+ tumors to have significantly worse OS than pN0 tumors (Figure 2B), which remained significant after adjustment for other variables (HR 2.08, p=0.012).



The Results section has been revised as follows (Page 6, lines 186-192):

"No significant difference in OS was found between the three primary tumor sites (p=0.17, Figure 2A). Median OS was 52 months for IHCCA, 32 months for EHCCA, and 50 months for GBC. Nodal positivity was significantly associated with poor OS with median OS of 56 months

for pN0 patients compared to 33 months for pN+ patients (p=0.0003). This association remained statistically significant in multivariable Cox regression (HR 2.08, p=0.012). Receipt of chemotherapy (HR 2.12, p=0.015) and chemoradiotherapy (HR=0.52, p=0.24) were also significantly associated with OS."

Comment 4: Adjuvant therapy after resection is an important topic. Please include data of regimen i.e. platinum-based, fluorouracil-based, gemcitbaine etc

Reply 4: Thank you for this comment. Although we did not analyze specific regimens due to small numbers within each group and the retrospective nature of the study, we have added this information to the Results section of our manuscript.

Page 5, lines 162-164:

Known chemotherapy regimens included gemcitabine-cisplatin (n=22), gemcitabine only (n=29), 5FU/capecitabine (n=10), FOLFOX/XELOX (n=14), and FOLFOXIRI (n=1).