Part A Point-by-Point Response

Part A: Point-by-point response to reviewers

Reviewer #1: I think that the manuscript is well written. I ask the authors to read my suggestions and to include a few important facts in the introductory part of the paper. I wrote in which direction to discuss. After that the paper could be accepted for publication. What are the new hypotheses that this study proposed? What are the new phenomena that were found through experiments in this study? What are the new concepts that this study proposes? What are the new methods that this study proposed? What are the limitations of the study and its findings? What are the future directions of the topic described in this manuscript?

Answer: Thank you for your suggestion, While we agree with your opinion,and our answer as followed:

(1) What are the new hypotheses that this study proposed?

Answer: This study proposed the new hypotheses that our study is the largest sample retrospectively compared the clinical epidemiological characteristics between IPNB, IPMN and traditional changiocarcinoma,Up to now there are no similar epidemiological studies in the literature.Moreover,The total of 1635 invasive IPNB cases is the largest multicenter retrospective cohort study.

(2) What are the new phenomena that were found through experiments in this study?

Answer: We concluded that the incidence of IPNB and IPMN decreased year by year, while the incidence of traditional changiocarcinoma increased year by year.The prognosis of invasive IPNB was not only regarding tumor grade and SEER historic stage, but also for different sites and tumor subtypes. Surgery and chemotherapy are associated with improved invasive IPNB outcomes; individuals who do not undergo surgery have the highest risk of death.

(3) What are the new concepts that this study proposes?

Answer: The prognoses of individuals with invasive IPNB were better than for individuals with invasive IPMN and traditional cholangiocarcinoma.we provided clear evidence that adjuvant chemotherapy can improve OS and CSS rates in individuals with invasive IPNB. The majority of invasive IPNBs occurred in the perihilum and AV. Tumor subtype was another important factor related to OS and CSS,For the non-mucin subtype of invasive IPNB were better than for individuals with mucin subtype of invasive IPNB.

(4) What are the new methods that this study proposed?

Answer: We used the Annual percentage changes (APCs) of incidence and incidence-based (IB) mortality and joinpoint regression analysis program ,We also used linear-by-linear association tests to evaluate the trends in the ordinal data, which provides a meaningful measure of ordinal variables.APC is a method of describing incidence or mortality trends over time by showing slope gradients or directions for each straight segment.

(5) What are the limitations of the study and its findings?

Answer: We admitted that the limitations of our study is that : First, studies using the SEER database involve a retrospective design, and the registries contain data for individuals from different institutions and time periods. Second,According to 2019 WHO proposal, intraductal papillary neoplasm of Ampulla are not included in IPNB.Since our study included the data from 1975 to 2016, and site the standard(site recode ICD-O-3/WHO 2008 and TNM7/CSv0204+schema), The ampullary IPNB in our study mainly be originated from the biliary tracts. Additionally, the database lacks central reviews by professional pathologists. Last, the study cohort lacked detailed information regarding

tumor recurrence, and palliative surgical methods and chemotherapy regimens, which have considerable OS and CSS impact.

In our study finding concluded that the prognosis between mucin and non-mucin subtypes differed significantly, which also indicated that the expression of mucin is related to the subtypes of IPMN and IPNB.Importantly, we also detected correlations between tumor type and location. The minority of invasive IPNBs (6.1%) occurred in the liver, and the majority occurred in the perihilar region (36.6%) and the hepatopancreatic AV (49.7%). This is because IPNB may originate from biliary stem/progenitor cells, which are located mainly in the peribiliary gland of the perihilum and the hepatopancreatic ampulla.And the incidence of invasive IPNB and IPMN decreased year by year, while the incidence of traditional changiocarcinoma increased year by year.The prognosis of invasive IPNB is better than invasive IPMN and traditional changiocarcinoma.

(6) What are the future directions of the topic described in this manuscript?

Answer: The inspiration of this article is that we found a rare case of hepatic mucoidepidermoid carcinoma(HMEC) in our cancer research center[1] *(published :Diagn Pathol. 2021 Apr 8;16(1):29. doi: 10.1186/s13000-021-01086-3)* , and found that the malignancy mucinous carcinoma in liver including IPNB,MCN (mucinous Cystadenocarcinoma) and adenosquamous carcinoma. So in our future research direction, we will analyze IPNB cases' tissues from our center by the next generation sequencing , combined with our published article to analyze the relationship between IPNB and HMEC.

Reference 1. Hou P, Su X, Cao W, Xu L, Zhang R, Huang Z, Wang J, Li L, Wu L, Liao W. Whole-exome sequencing reveals the etiology of the rare primary hepatic mucoepidermoid carcinoma. Diagn Pathol. 2021 Apr 8;16(1):29. doi: 10.1186/s13000-021-01086-3

Reviewer #2: The original findings of this manuscript are the current population-based study revealed a gradual decrease in the incidence and IB mortality rates of invasive IPNB in the US population during 1975–2016. the new hypotheses are not that this study proposed the quality and importance of this manuscript are to IPMN is to evaluate the incidence according to historical records. İt can not solve the questions/issues about in this topic.

Answer: Thank you for this comment, Firstly, there is a current lack of standardization of researches in rare tumors;Second,our team had published rare tumor(Gastrointestinal mixed adenoneuroendocrine carcinoma) through the SEER database, and have been highly recognized by the academic community[1].Moreover,There were in total of 19 articles reported in literatures about IPMN which is the counterpart of IPNB downloaded from SEER databases, and the APC of IPMN in our study was consistent with reported literature.

Reference 1. Wang J, He A, Feng Q, **Hou P,** Wu J, Huang Z, Xiao Z, Sun C, Liao W, Wu L. Gastrointestinal mixed adenoneuroendocrine carcinoma: a population level analysis of epidemiological trends. J Transl Med. 2020 Mar 14;18(1):128. doi: 10.1186/s12967-020-02293-0. PMID: 32169074; PMCID: PMC7071749.

Reviewer #3 This is a well-written paper showing the epidemiology and outcomes of IPNB. The comparison between IPNB, cholangiocarcinoma and IPMN will be of interest to readers.

Answer: Wow, we are aspired by the reviewer' insightful approval of our manuscript, and this would be a superexcellent encouragement for our future work.

Reviewer #4 Although the paper is well-written, I have several questions and concerns for the authors: Abstract Please mention the total number of IPNB cases. Introduction Please cite relevant studies recently published (https://doi.org/10.1016/j.hpb.2019.11.007; https://doi.org/10.3390%2Fjcm9123991; https://doi.org/10.1177/0300060518792800). Methods Which variables did you adjust Cox regression for? Please clarify the reasons you selected R packages for statistical analysis. Results How did you precisely record the information on radiation and chemotherapy? On what basis did you select 68 years as the cut-off for age? In Table 2, please specify "others" for Race. Discussion Please allude to the association of IPNB and its outcome with genetic mutations. The references are not written according to the journal's style. Footnotes Please state parts related to this section, conflict-of-interest statement, ethics, etc.

Answer: Thank you very much,the referee comments would be constructive and helpful for us.We describe this in the revised manuscript in **Abstract (Underline57-58),**

Relevant studies recently published have been cited in *Introduction* section, paragraph 2 (Underline 89-90), paragraph 3 (Underline 97-98),*and Materials and Methods* section(Underline 172-178), Satistical Snalyses (Underline 197-198),*Deleted the sentence(* … using R software ……***),*respectively.**

1.Which variables did you adjust Cox regression for.

Answer: Independent predictors of mortality were determined by Cox proportional hazard regression. The variables analysed included patient age, site, tumour grade, stage,mucin classification and treatment.The detail variables and results were shown in the forest map(Figure 5) .

2.Please clarify the reasons you selected R packages for statistical analysis.

Answer: We apologize for our little mistake, we just use R packages to draw forest map after using the results of Cox proportional hazard regression model in the multivariable analysis, and do not use R language for statistical analysis. So we deleted this sentence in the revised manuscript.

3.How did you precisely record the information on radiation and chemotherapy?

Answer:SEER database concluded RADIATION RECODE and CHEMOTHERAPY RECODE.as followed:Radiation recode (*Beam radiation;Radiation,NOS, method or source not specified;Recommended,unknown if administered,Radioisotopes*) .Radiation sequence with surgery recode (*No radiation and/ or cancer-directed surgery;Radiation after surgery;Radiation before and after surgery ,Radiation prior to surgery,sequence unkown,but both were given*).Chemotherapy recode (*yes, no/unk*).

4.On what basis did you select 68 years as the cut-off for age?

Answer: Because age is a prognosis of OS and CSS by the Kaplan−Meier method and the log-rank test ,and the median age of 1635 patients with IPNB was 68 years old, so we select 68 years old as cut-off for age.

5.In Table 2, please specify "others" for Race.

Answer: Thank you very much,other race include: American Indian/Alaskan Native, Asian/Pacifc Islander,details in the revised manuscript table 2 annotations (***line 570 and 574).***

6.Discussion Please allude to the association of IPNB and its outcome with genetic mutations.

Answer: As mentioned above, the ideal in our manuscript about IPNB came from our clinical discovery of a rare primary hepatic mucoepidermoid carcinoma(HMEC) in our department, it is concluded that the main GNAS R201 mutation resulted in mucin in HMEC,The article has now been published [1] ,so we cited this part in the discussion section.

References 1 Hou P, Su X, Cao W, Xu L, Zhang R, Huang Z, Wang J, Li L, Wu L, Liao W. Whole-exome sequencing reveals the etiology of the rare primary hepatic mucoepidermoid carcinoma. Diagn Pathol. 2021 Apr 8;16(1):29. doi: 10.1186/s13000-021-01086-3

7.References The references are not written according to the journal's style. Footnotes Please state parts related to this section, conflict-of-interest statement, ethics, etc.

Answer: The references are written according to the journal's style. We modified and added this section .Footnotes state parts related to this section, conflict-of-interest statement, ethics, etc,were added.

Part B: Point-by-point response to Editor

1) Because of the anatomical proximity of the pancreas and the bile duct, the simultaneous development of the foregut endoderm, the peribiliary gland containing pancreatic exocrine acini, and biliary stem cells in the peribiliary glands can differentiate into cholangiocytes as well as hepatocytes or pancreatocytes. There are confusing explanations as the peribiliary gland containing pancreatic exocrine acini.

Answer: Thank you for your suggestion,we couldn’t agree more.So we revised that the sentence ***“the peribiliary gland containing multipotent stem cells in biliary tract can differentiate into cholangiocytes as well as hepatocytes or pancreatocytes”*** .in

revised manuscript.**Study population section (underline 83-85).Results section line 232 (underline) and line 356-358 (underline).Of course,we also revise the table 1 (underline) and figure 4A**

2) We recorded the following demographic and clinicopathological variables: sex, age at diagnosis, year of diagnosis, race, detailed tumor site according to the tumor-node-metastasis (TNM)7 and Cancer Staging schema, version 0204, histological subtype, SEER historic stage, grade, surgery, radiology, chemotherapy, survival (in months), vital status recode, and cause-specific death. There are radiology . What does you mean?

Answer :We apologize for our misuse of words. The correct expression should be "radiotherapy",and we revised this word in **Materials and methods** ***(Study population Underline 197-199).*** **Results section (underline 238,230) and (underline 232-233).Of course,we also revise the table 1 (underline) and figure 4A**

3) However, the median OS and CSS of individuals with invasive IPMN was the worst at only 6 months (95% CI: 5.8–6.2 months) and 9 months (95% CI: 8.6–9.4 months), respectively, and the 1-, 3-, and 5-year OS and CSS rates were 30.3% and 39.5%, 11.5% and 18.8%, and 8.4% and 15%, respectively. The median OS and CSS of individuals with traditional cholangiocarcinoma was 10 months (95% CI: 9.7–10.2 months) and 15 months (95% CI: 14.6–15 months), respectively; and 1-, 3- and 5-year OS rates were 43.9% and 54.1%, 18.3% and 28.6%, and 12.4% and 22.2%, respectively there are better OS and CSS of IPMN than OS and CSS of cholangiocarcinoma. this independent cohort should be supported.

Answer :So sorry,we repeatedly confirmed the previous manuscript, and our results still turned out to be "there are bad OS and CSS of invasive IPMN than OS and CSS of traditional cholangiocarcinoma". So we're not sure how to interpret this sentence, **“this independent cohort should be supported. ”**

4) Interestingly, both the OS and CSS of the mucin and non-mucin subtypes of invasive IPMN were also statistically significant. what does you mean in sentence?

Answer :Invasive IPMN and Invasive IPNB share the same clinicopathological features. and even biological behaviors.According to the previous result in our manuscript,similar decreases in IPNB incidence and IB mortality were seen in invasive IPMN but not for traditional cholangiocarcinoma, and both the OS and CSS of the mucin and non-mucin subtypes of invasive IPNB and IPMN were statistically significant, further indicated that IPNB is a counterpart of IPMN,originated from the same pathogenesis .

5) We evaluated the incidence and IB mortality associated with invasive IPMN of the pancreas, which may represent a carcinogenic pathway different from the traditional carcinogenic pathway of cholangiocarcinoma caused by flat atypical hyperplasia. You mention refrence 14. it does not has that.

Answer :We apologize for our misuse of reference disorder. The correct refrence should be "refrence 18",and we revised the reference.

6) **We found that ampullary invasive IPNB accounted for 47.9% of the individuals in our study cohort. Cholangiocarcinomas does not include ampullary invasive IPNB.**

Answer :Thank you very much for the kind reminder,this comment would be constructive and helpful for us.As we all known,based on the anatomical site of origin, Cholangiocarcinomas(CCA) is classified as intrahepatic cholangiocarcinoma (iCCA), perihilar CCA (pCCA), and distal CCA (dCCA). The iCCAs are classifified into mass-

forming, periductal-infifiltrating, and intraductal growth, and pCCAs/dCCAs are classifified into flat- and nodular-infiltrating, and papillary types.CCA can generate from epithelial cells in the biliary tracts and multiple stem cell which are found in peribiliary glands(PBGs) within extrahepatic and large intrahepatic bile ducts[1]. In adults, PBGs predominantly occur at branching points of the biliary tree and are most numerous at the hepatopancreatic ampulla[2].PBGs in the hepatopancreatic ampulla attracted attention as a potential origin of CCA and IPNB[3,4](Fig1).In fact, Four morphological IPNB subtypes exist-intestinal, pancreaticobiliary, gastric, and oncocytic,the highest rates of malignancy have been demonstrated for pancreaticobiliary[5] .The pancreaticobiliary subtype is more frequently associated with the presence of invasive malignancy[6].

Of course,we acknowledged that according to 2019 WHO proposal, intraductal papillary neoplasm of Ampulla are not included in IPNB. In fact,our data is from 1975 to 2016, we first filtered site recode ICD-O-3/WHO 2008 (Definition:Liver C220 , intrahepatic Bile Duct C221, other Biliary C240-C249) in the **methodological screening(underLine129-132)**.Subsequently,We collected TNM7/CSv0204+schema site information from 1975 to 2016.Therefore,the ampullary IPNB cases in our cohort study mainly be originated from the biliary tracts. With regard to the fact that we have modified the methodology and results of this section, we have also added this part to the discussion section.We simply describe it as ampullavater bile may be misunderstood,and ampulla vater bile can be defined as hepatopancreatic ampulla bile or pancreaticobiliary ampulla precisely.So we revised “ampulla vater bile” as “pancreaticobiliary ampulla” in our manuscript.

1. Carpino G, Cardinale V, Onori P, Franchitto A, Berloco PB, Rossi M, et al. Biliary tree stem/progenitor cells in glands of extrahepatic and intraheptic bile ducts: an anatomical in situ study yielding evidence of maturational lineages. J Anat 2012; 220:186-199.))

2. Cardinale V, Wang Y, Carpino G, Cui CB, Gatto M, Rossi M, et al. Multipotent stem/progenitor cells in human biliary tree give rise to hepatocytes, cholangiocytes, and pancreatic islets. HEPATOLOGY 2011;54:2159-2172.

3. Cardinale, V.; Wang, Y.; Carpino, G.; Reid, L.M.; Gaudio, E.; Alvaro, D. Mucin-producing cholangiocarcinoma might derive from biliary tree stem/progenitor cells located in peribiliary glands. Hepatology 2012, 55, 2041–2042.

4. Nakagawa H, Hayata Y, Yamada T, Kawamura S, Suzuki N, Koike K. Peribiliary Glands as the Cellular Origin of Biliary Tract Cancer. Int J Mol Sci. 2018 Jun 12;19(6):1745. doi: 10.3390/ijms19061745.

5. Kubota K, Nakanuma Y, Kondo F, Hachiya H, Miyazaki M, Nagino M, Yamamoto M, Isayama H, Tabata M, Kinoshita H, Kamisawa T, Inui K. Clinicopathological features and prognosis of mucin-producing bile duct tumor and mucinous cystic tumor of the liver: a multi-institutional study by the Japan Biliary Association. J Hepatobiliary Pancreat Sci. 2014 Mar;21(3):176-85. doi: 10.1002/jhbp.23

6. Adsay NV, Merati K, Basturk O, Iacobuzio-Donahue C, Levi E, Cheng JD, Sarkar FH, Hruban RH, Klimstra DS. Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms: delineation of an "intestinal" pathway of carcinogenesis in the pancreas. Am J Surg Pathol. 2004 Jul;28(7):839-48. doi: 10.1097/00000478-200407000-00001

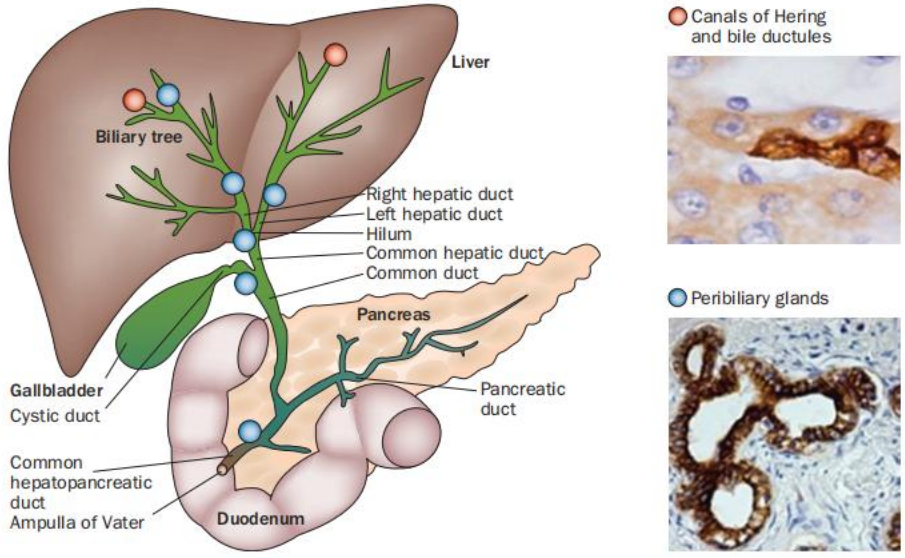

Figure 3 | Stem cell niches in the intrahepatic and extrahepatic biliary tree. Stem cell niches within the liver are located in the canals of Hering (red circles). Hepatic stem cells are precursors to hepatoblasts, which are presumed to be the transit-amplifying cells that first give rise to committed progenitors, and then to hepatocytes and cholangiocytes.[19] Peribiliary glands contain stem cell niches within the biliary tree (blue circles).[31] These glands are along the biliary tree from the hepatopancreatic common duct near the duodenum up to the septal ducts. High numbers of peribiliary glands occur in the cystic duct, hilum and periampular regions, sites that are vulnerable to oncogenic transformation. Since progenitor and/or stem cells within peribiliary glands probably act as sources for cell turnover of the entire biliary tree distal to the interlobular bile ducts, transformation of hepatic or biliary stem cells can give rise to tumors composed of heterogenous cellular phenotypes. Insets show immunohistochemistry staining for CK-7. Adapted from Cardinale, V. *et al. World J. Gastrointestinal. Oncol.* **2**, 406–416 (2010), which is published under a Creative Commons Attribution License by Baishideng Publishing Group.

Cited by **Cardinale V, Wang Y, Carpino G, Mendel G, Alpini G, Gaudio E, Reid LM, Alvaro D. The biliary tree--a reservoir of multipotent stem cells. Nat Rev Gastroenterol Hepatol. 2012 Feb 28;9(4):231-40. doi: 10.1038/nrgastro.2012.23**

Finally, we would like to add new thanks, We apologize for missing this from the original manuscript. “We also thank Peng Huang, PhD., from the center for Evidence-Base Medicine,School of Public Health, NanChang University for professional knowledge guidance in statistics”.All authors agree with this change and we confirm that it does not affect our conflict of interest.