Cover Letter

Dear editor and reviewers,

Thank you very much for reviewing our manuscript “Malignancy risk factors and prognostic variables of pancreatic mucinous cystic neoplasms in Chinese patients” and for providing such valuable comments. These insightful comments helped us improve the current version of our manuscript. We have carefully considered the comments and tried our best to address every one of them. We hope this carefully revised manuscript meets your high standards.

Below, we provide point-by-point responses. All modifications in the manuscript have been highlighted with a yellow background.

Sincerely,

Nan Li, PhD

Professor of Pathology

Response to Editor’s comments

[Editor’s comments] Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Please authors are required to provide standard three-line tables, that is, only the top line, bottom line, and column line are displayed, while other table lines are hidden. The contents of each cell in the table should conform to the editing specifications, and the lines of each row or column of the table should be aligned. Do not use carriage returns or spaces to replace lines or vertical lines and do not segment cell content. Please check and confirm whether the figures are original (i.e. generated de novo by the author(s) for this paper). If the picture is ‘original’, the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT): Copyright ©The Author(s) 2022. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight

articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/.

Response: Thank you for your prompt and detailed review of our manuscript. We have modified the figures and tables according to your instructions. We have provided the revised figures and tables as separate files in the attachments. In addition, we have added an "Article Highlights" section to the manuscript, which summarizes our key findings and contributions in a concise and reader-friendly format. We also took into account the reviewers' recommendations and utilizedthe Reference Citation Analysis (RCA) to further refine the content and increase our study's accuracy, validity, and reliability. Thank you once again for your feedback, which has helped us improve the quality and impact of our study.

Response to Reviewer 1

[General Comment] Thank you for you manuscript. I found it very interesting, with a direct impact in clinical practice. Pancreatic cystic neoplasms are more commonly diagnosed due to better imaging techniques but accurate characterization and management is not so straightforward. Standardized nomenclature and classification is essential. Precise radiology reports are lacking and many times mucinous neoplasms are only diagnosed post-operatively.

Response: Thank you very much. By the way, we would like to apologize for the inconvenience caused by the lack of line numbers and page numbers in our manuscript. As the manuscript was generated through the journal website system, these features were unfortunately not included. Therefore, we kindly request that you manually insert the line and page numbers in the revised manuscript as necessary. Furthermore, we have included screenshots of the revised manuscript to ensure that our modifications are clear and easily verifiable. These screenshots can be found beneath each corresponding comment.

suggestions:

[Comment 1] Related to this, did all your patients have a CT scan, or some of them performed MRI / endoscopic US? Preferred imaging modality could be an interesting topic to review in your patients.

Response: All patients included in our study underwent preoperative imaging examinations. All patients had CT scans performed, and additional MRI/endoscopic US was performed as needed. Therefore, some patients underwent MRI/endoscopic US after the CT results were available. We have added a comparison of the detection rates of these three imaging modalities to the results section [Pg12, Ln2-7]. Unfortunately, the results showed no statistically significant difference. In addition, we have added a discussion of the imaging modalities to the discussion section [Pg22, Ln11-20; Pg23,Ln1-15].

lesions (PCLs) and the use of imprecise terms such as pancreatic cystic or solid masses. The preoperative imaging modalities employed in this study comprised computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS). Of the 48 patients, all underwent CT, 33 underwent MRI, and 21 underwent EUS. The corresponding detection accuracy rates were 64.6%, 87.9%, and 71.4%, respectively. Nevertheless, there was no significant difference in diagnostic accuracy among the three methods (*P*=0.64).

In addition, preoperative imaging evaluation of various risk indicators for MCNs seems to require the use of different modalities. No single test can accurately diagnose all cases, and in fact, most patients undergo more than one diagnostic procedure[28]. Therefore, the comprehensive use of different detection methods is more conducive to preoperative diagnosis. In our study, this may be attributed to the preference of imaging doctors at our hospital. CT is the preferred initial test for patients undergoing physical examination or with symptoms, and if the CT results are inconclusive or require further confirmation, MRI or EUS will be performed. Unfortunately, there was no significant difference between these three detection methods in our study; nevertheless, combining CT with MRI/EUS

increased the preoperative diagnosis rate of our patients from 64.4% to 72.9%. A review of the literature suggests that MRI has slightly higher accuracy in distinguishing between malignant PCNs and benign lesions than CT[29]. MRI was found to be more effective than EUS in distinguishing malignant MCNs[30]. CT combined with MRI was shown to be better than CT alone in the preoperative diagnosis of pancreatic cysts[31]. Moreover, patients with MCNs who have a certain risk of malignant transformation need long-term monitoring or even lifelong monitoring until surgery is no longer a suitable treatment option. For long-term follow-up of MCNs, MRI is the preferred method[18]. This may be because MRI has high contrast resolution and does not involve the use of radiation, which may increase the risk of developing malignant tumours in patients with long-term exposure to CT. EUS is an invasive procedure that highly depends on the operator's skills, especially when combined with aspiration for cyst fluid analysis. EUS is recommended for cysts with significant risk characteristics or when a more accurate diagnosis may change the patient's treatment plan[32].

[Comment 2] Also, after performing image review was the conclusion the same as in the pre-operative report?

Response: All patients included in our study were confirmed by postoperative pathology diagnosis. Imaging results of all patients for the past ten years were reviewed again by radiologists, which significantly reduced the misdiagnosis rate. This was mainly due to the development of imaging technology, in-depth research on MCNs in the medical field and, subsequently, the improvement in the understanding of MCNs by radiologists at our hospital. However, the diagnostic rate of the secondary review of the imaging results was still low, at only 72.9%. In addition, the radiologists carefully evaluated the imaging characteristics of each patient's mass, such as duct dilatation and septations, which may have been overlooked in the initial preoperative imaging examination.

[Comment 3] Surgical data is somewhat lacking. Surgical intervention could be added but mostly R status is important when considering malignant MCNs; lymph node harvest could also impact prognosis.

Response: Depending on the location of the mass in the pancreas, the surgeon chose the optimal surgical approach. The masses were sent for postoperative pathology examination, and no residual cancer cells were found under the microscope at the margins, indicating R0 status.

In addition, lymph node sampling was performed during surgery for each patient, and the pathology showed that all patients had negative lymph nodes. The R0 status of the mass and negative lymph nodes have been added to the text [Pg15, Ln2-5]. Although R status and lymph node involvement have important implications for prognosis, due to the limitations of our cases, we unfortunately could not further explore these factors.

Consequently, the clinicopathological characteristics of the 11 MCN–AIC patients were further explored (Table 2). The tumours examined in this study did not display any signs of lymph node involvement, distant metastasis, or nerve invasion. In addition, all the tumours were successfully resected, and the margins were negative. Based on the AJCC cancer staging system[6], all 11 cases of MAC–AIC

[Comment 4] Your manuscript is missing some items in the STROBE guidelines (for example, title should indicate article type, inclusion / exclusion criteria are not specified, etc...) and the checklist should be completed and added to the submitted files.

Response: Thank you for these reminders. We revised the sentence as follows: We have added the article type above the title [Pg1, Ln5]. The inclusion and exclusion criteria have also been added to the manuscript [Pg8, Ln13-19]. We have completed the STROBE checklist and submitted it along with the manuscript.

Retrospective Study

Inclusion and Exclusion Criteria

The inclusion criteria were patients who had been confirmed by surgical pathology to have MCN, underwent imaging examinations, and did not receive preoperative radiotherapy or chemotherapy. The exclusion criteria included poor-quality radiographic images, insufficient pathological diagnostic data, the presence of other pancreatic diseases, and a history of other malignant tumours. These criteria were carefully selected to ensure the accuracy and reliability of the study's findings.

other comments in the attached file:

[Comment 1] one typographic error page 8 - LGD instead of HGD：

Response: Thank you very much for the reminder. We have made revisions accordingly [Pg12, Ln10].

Postoperative pathological diagnosis revealed 36 cases of LGD, 1 case of HGD,

[Comment 2] Do you know the cause of death? Was it disease progression? Adding disease free survivals here and not only in your discussion would better demonstrate prognosis in malignant cases. Also, were all resections R0?

Response: Thank you for your comment. We apologize for any confusion caused by our unclear phrasing. We would like to clarify that all patients, except for one who died from a stroke, had tumour-related

deaths. We have revised the phrase "the 5-year survival rate" to "the 5-year disease-specific survival rate" to better reflect our findings [Pg14, Ln18-19]. Additionally, we confirmed that complete tumour resections were performed with negative margins, resulting in R0 status.

18 malignant MCNs, 3 died at 8, 14, and 46 mo after surgery; the 5-year disease-
19 specific survival rate was 70.1%. All deaths occurred in MCN-AIC patients.

[Comment 3] TNM stage is not expected to show significant prognostic differences between stages IA / IIA with a sample of 12 patients.

Response: It might be unclearly expressed that all the malignant MCN cases we studied were at an early stage, including only stages IA and IB and not stage IIA. Some previous studies have reported higher survival rates for IA versus IB PDAC patients. However, little research has investigated the survival differences between invasive PDAC in the IA and IB subgroups of MCN-AIC. Therefore, we examined whether TNM staging served as a prognostic factor for early-stage MCN. Our statistical analysis revealed no relationship between them.

[Comment 4] I find this affirmation poorly substantiated. Maybe "Diligent monitoring might be preferred…" could be an alternative.

Response: Thank you very much for the suggestion. We have made revisions accordingly [Pg26, Ln19].

regardless of whether adjuvant therapy was offered. Diligent monitoring might be preferred over intensive systemic therapy for stage I MCN-AIC with encapsulated invasion.

[Comment 5] Current guidelines also support FOLFIRINOX as first line systemic therapy in PDAC (for example, NCCN)

Response: Thank you very much for the reminder. We have made revisions accordingly [Pg25, Ln17-20].

For patients with resected pancreatic cancer, the current standard of care mandates the administration of either a modified FOLFIRINOX (mFOLFIRINOX) or a gemcitabine-based regimen, provided that no contraindications are present[40]. However, limited research has been conducted on the benefits and risks

Response to Reviewer 2

[General Comment] In this single center retrospective study, the authors review the characteristics and outcomes of patients who underwent a pancreatic resection for MCN and investigated the factors associated with an increased risk of malignancy (invasive carcinoma or with atypical hyperplasia) associated with MCN (MCN-AIC) and with a decreased oncologic related survival among patients affected by MCN-AIC. this is a well written manuscript focused on a pancreatic disease rare and as such scarsely studied and investigated.

Response: Thank you very much for your comments, which helped us improve this manuscript. By the way, we would like to apologize for the inconvenience caused by the lack of line numbers and page numbers in our manuscript. As the manuscript was generated through the journal website system, these features were unfortunately not included. Therefore, we kindly request that you manually insert the line and page numbers in the revised manuscript as necessary. Furthermore, we have included screenshots of the revised manuscript to ensure that our modifications are clear and easily verifiable. These screenshots can be found beneath each corresponding comment.

suggestions:

[Comment 1] I suggest the authors to avoid using an acronym before reporting the extended version of the term the acronym refers to (see in the abstract: PR).

Response: Thank you very much for the reminder. We have made revisions accordingly[Pg4, Ln19].

19 type stroma verified by progesterone receptor staining were included. Preoperative

[Comment 2] First line of the paragraph "Microscopical and immunohistochemical features", "1 case of LGD" should be "1 case of HGD".

Response: Thank you very much for pointing this out. We have made revisions accordingly [Pg12, Ln10].

10 Postoperative pathological diagnosis revealed 36 cases of LGD, 1 case of HGD,

[Comment 3] Paragraph "survival analysis and prognostic variables of MCN-AIC": starting from line 11 to 20: this section is not very clear to me: I suggest to rephrase the first sentence.

Response: Thank you very much for pointing this out. We have rephrased this section to improve its clarity [Pg15, Ln2-11].

were further explored (Table 2). The tumours examined in this study did not display any signs of lymph node involvement, distant metastasis, or nerve invasion. In addition, all the tumours were successfully resected, and the margins were negative. Based on the AJCC cancer staging system[6], all 11 cases of MAC–AIC were categorized as stage I, with 7 patients having stage IA cancers and 4 having stage IB cancers, depending on the tumour size. The encapsulated invasion of MCN–AIC was defined as infiltrating components not exceeding the outermost layer of the capsule, with or without infiltration into the subepithelial stroma or cystic septa[7]. Using this definition, 9 tumours were encapsulated, while 3 were extracapsular (Cases 1–3). In Case 1 and Case 2, the cancerous tissue extended

[Comment 4] In addition, It is not clear which patients the authors are referring to when they speak about when they report on CASE 1, 2 and 3. Are they the patients who died?

Response: Thank you very much for pointing this out. We assigned unique identifiers (Case 1-Case 11) to the 11 MCN-AIC patients (Table 2). Cases 1, 2, and 3 all exhibited invasive cancer with infiltrating components breaking through the capsule; thus, we provided detailed descriptions of their pathological features. Additionally, all three patients

experienced tumour-related deaths. We have revised this section of the manuscript for clarity[Pg15, Ln9-11].

Table 2. Characteristics of 11 patients with MCN-AIC

Case	Age (yr)	Sex	Tumor size(cm)	Largest dimension (cm)	Invasion Pattern	pT stage	pTNM Stage	Status	OS(mo)	Adjuvant chemotherapy
1	72	Female	8	3	Extracapsular	T2	IB	Death	8	Yes
2	60	Male	14	1.2	Extracapsular	T1	IA	Death	14	Yes
3	57	Male	7.2	4	Extracapsular	T2	IB	Death	46	No

layer of the capsule, with or without infiltration into the subepithelial stroma or cystic septa[7]. Using this definition, 9 tumours were encapsulated, while 3 were extracapsular (Cases 1–3). In Case 1 and Case 2, the cancerous tissue extended

[Comment 5] the discussion is very interesting but also very long: I suggest the authors to shorten it, for example byremoving/shortening the paragraphs concerning the risk factors (for malignancy) whcih are known in the literture but were not confirmed in the curtrent study.

Response: Thank you very much for the reminder. We appreciate your suggestion to shorten the discussion section. Based on your

recommendation, we have removed paragraphs that refer to risk factors that have been identified in previous literature but could not be confirmed in our study. We believe this streamlines the discussion section without sacrificing any important information.

[Comment 6] a study limitation paragrph is lacking in the discussion: among eventual limitations, the small number of patients included in the current study should be highlighted.

Response: Thank you for drawing our attention to the need for a limitation paragraph in the discussion. We have added an appropriate paragraph that highlights the small sample size of our patient cohort as a limitation of our study. We also addressed other limitations related to the methodology [Pg27, Ln16-20; Pg28, Ln1-3].

16 This study was subject to several limitations. First, it was a retrospective study that
17 only included patients who underwent surgical resection and were pathologically
18 confirmed to have MCNs, which may result in selection bias. Second, the rarity of
19 MCNs limited the sample size, leading to a large confidence interval that may have
20 hindered statistical analysis. Third, the MRI/EUS results were based on previous CT

information, potentially affecting the interpretation of imaging results. Therefore, further multicentre and large-scale studies are needed to explore the clinical, pathological, imaging, and biological behaviours of MCNs.

[Comment 7] I suggest the authors to comment on how this manuscript may impact on clinical practice: the risk factors for malingnant MCN are quite similar to those of IPMN... Thus, should we manage MCN similar to IPMN? please comment on this.

Response: Thank you for the interesting question about the potential clinical impact of our study on MCN management. We agree that the risk factors for malignant MCNs identified in our study are similar to those identified for IPMNs. However, we believe that the management of MCNs should not be similar to that of IPMNs. Based on your suggestion, we have added a new paragraph to the discussion section to explore this topic further [Pg20, Ln11-20; P21; Pg22, Ln1-10].

The management of malignant pancreatic tumours is predominantly centred on postoperative follow–up, whereas the management of MCNs places greater emphasis on the potential risks associated with preoperative monitoring and misdiagnosis. The management plan for IPMN is relatively mature, considering that dedicated management guidelines for IPMN have been published[1]. However, despite MCN being a precursor lesion of pancreatic cancer, similar to IPMN, the management plan for MCN remains unclear, particularly in identifying high–risk factors. Although the risk factors and surgical indications for IPMN and MCN are not differentiated in some guidelines[4], the demographic, cystic, histological and other characteristics of these two tumours differ, so they should

be managed differently, particularly with respect to exploring risk factors. Unfortunately, our findings suggest that the risk factors for malignant MCNs are quite similar to those for IPMNs. The size of the lesion and the presence of solid components/wall nodules have been widely recognized as high-risk factors for malignant transformation of MCNs and IPMNs in various guidelines and publications, including our study. While various other malignant risk factors for MCN reported in the literature are statistically significant, they require further confirmation. In the case of IPMNs, the characteristic risk factor is main pancreatic duct dilation, especially when the diameter exceeds 10 mm, given that IPMNs grow within the pancreatic ducts. However, MCNs lack their own characteristic risk factors. Based on their relationship with the pancreatic ducts, IPMNs are classified into the main duct type, mixed duct type, and branch duct type, and direct surgical resection is recommended for the first two types due to a high risk of malignant transformation[18]. However, immediate surgical intervention for MCNs with risk factors remains controversial. Höhn *et al* conducted a retrospective analysis exploring the risk factors for malignant transformation of MCNs, similar to our own analysis[14]. They recommended radical resection surgery for all eligible patients suspected of having MCNs due to concerns about the potential risk of malignant progression and the level of expertise of pancreatic surgeons in low-volume centres. However, this view seems too radical, and the decision for MCN patients

to undergo surgery should be more cautious, as the incidence and malignant transformation rate of MCNs are much lower than those of IPMNs[27]. The main argument for surgical resection in all MCN patients based on eliminating the risk of future malignancy seems invalid. It is essential to increase awareness and continuously conduct research on this rare tumour, especially in terms of preoperative malignant risk factors. Based on the above premise, a multidisciplinary team with expertise in pancreatic cysts and surgery can combine various reported risk factors, patient comorbidities, surgery–related complications, and mortality rates to comprehensively evaluate the potential risks and benefits of surgery and monitoring, thereby making the best treatment decision for the patient.