

Peer Review File

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Comment 1: There are multiple grammatical errors in the discussion (ie. extra commas (line 189), incorrectly placed quotation marks (line 190 and 191, 309), missing periods (line 236) and commas (lines 225, 236, 283)

Reply 1: *Thank you for your detailed review of our manuscript.*

Changes in the text: A revision of the complete manuscript regarding spelling and grammar was performed by a native speaker.

Comment 2: The authors reported having a high number of consecutive patients in a single center. Was there a sample size calculation?

Reply 2: *Including 63 patients, our study presents the highest number of malignant MCN (16) with histologically proven ovarian stroma from a single center in the current literature. These 63 patients represent only a small fraction of > 4500 pancreatic surgeries done over the study period at our institution, which shows how rare this entity is. Even a high volume center wouldn't be capable of performing a well designed prospective study on MCN within a reasonable time frame. Thus, we did not perform a sample size calculation but rather aimed at inclusion and complete data assessment of every case available to us.*

Changes in the text: No changes.

Comment 3: The follow-up period of the cohort was not defined. Did you include patients who had less than 6 months' follow-up? How long was the follow-up period and what was the time to malignancy?

Reply 3: *We are really grateful to the reviewer for his suggestion to include follow-up data in the manuscript. All authors put tremendous efforts during the last two weeks to collect robust and complete follow-up data. This was a very arduous challenge, because of the legislative difficulties about collecting long-term data of patients for research purposes in Germany. We are very proud to have obtained all necessary data in this short time frame. Follow up time ranged between 2 to 180 months (mean 87 ± 55 months). Median follow-up was 84 months (IQR 35.5 – 140). A total of 17 patients were lost to follow-up after a median of 50 months (IQR 33.75-81.5), mean 59.88 ± 35.82 months, min-max 6-120 months. Actually, we didn't have any long-term survivors with less than 6 months follow-up. Only 1 patient was lost to follow-up after his control visit 6 months after surgery. Two other patients died 2 months after surgery – the first one died of multiple rapidly progressing brain metastases, which were not diagnosed before surgery due to failing symptoms; the second patient died in a rehab facility of fulminant hemorrhagic stroke.*

Changes in the text: Results of survival analysis and recurrence data were added on page 11 line 7 to page 11 line 18. Figure 4 was added, containing Kaplan-Meier survival curves. A figure legend was added on page 28, line 22-24. We added a brief discussion of our findings on survival data on page 18, line 2-14.

Comment 4: The authors mentioned that calcification, mural nodules and wall thickness were assessed as radiologic features. What was defined as thick walls? What about pancreatic duct dilatation?

Reply 4: *Wall thickness was defined as a cyst wall > 3 mm, according to current literature. In contrast to other tumorigenic lesions, dilatation of the pancreatic duct is not been demonstrated to be associated with malignancy in MCN. Opposite to IPMN, MCN do not have connection to the main pancreatic duct and rarely cause any duct dilatation.*

Changes in the text: We added the definition of wall thickness to our manuscript on page 6 line 21-22 and also included a brief statement regarding duct dilatation for further clarification at the same location.

Comment 5: Was there recurrence?

Reply 5: *None of the patients with benign MCN developed any recurrence during follow-up. However, one male patient underwent distal pancreatectomy in 2005 for benign MCN adenoma and then underwent completion pancreatectomy in 2019 because of pancreatic ductal adenocarcinoma in the region of the pancreatic stump! The patient underwent adjuvant chemotherapy and is still alive and doing well. Another female patient with distal pancreatectomy for benign MCN developed ovarian cancer 60 months later, but after successful treatment is currently still alive at 100 months follow-up.*

All patients with lethal outcome after surgery for invasive malignant MCN died of diffuse metastatic disease, apart from the one who died of stroke as mentioned above. There was only one recorded local recurrence in a female patient who underwent total pancreatectomy for a large malignant MCN. Despite refusing adjuvant chemotherapy she lived disease-free for 60 months after surgery before a local recurrence at the VMS was detected and then surgically removed. Numerous surgical procedures followed with removal of different localized metastases and the patient died 144 months after initial surgery.

Changes in the text: The above information was included in the results section of the manuscript in order to make follow-up data more transparent (page 11, line 19 to page 12, line 10). We also added table 5, summarizing our findings regarding follow-up, survival and recurrence.

Comment 6: Were there other post operative complications? The Clavien Dindo Grading was used. What is that?

Reply 6: *The Clavien-Dindo-Classification (CDC) was first published in 2004 by Dindo, Demartines and Clavien in Annals of Surgery and immediately gained international recognition due to its simplicity and generalizability. Since then it has been validated in numerous trials. CDC describes all postoperative complications, both medical and surgical and grades the according to the required treatment. CDC is widely accepted in surgical research for the description of postoperative outcomes. Occurrence of postoperative pancreatic fistula (POPF) is a typical complication of pancreatic surgery and of special interest for related research. Its classification by the International Study Group on Pancreatic Fistula (ISGPF) is also widely accepted in surgical research. Using the CDC and the ISGPF's definition we classified and described all postoperative complications. Following the reviewers suggestion we revised all cases of POPF and strictly*

applied the ISGPF definition to separate cases into biochemical leaks, grade B and grade C fistulas.

Changes in the text: Revised data regarding POPF was added to table 1 and to page 9 line 23 - page 10 line 2.

Comment 7: The authors did not find a correlation between median MCN size and malignancy. Was the mean size evaluated?

Reply 7: *As MCN can greatly differ in size and the compared subgroups in the study were small from statistical point of view, we intentionally used median values to compensate for a supposed not normal distribution. Nevertheless, mean size was evaluated and added to the text: Mean size was 44.09 mm (standard deviation: 29.425) for benign MCN and 52.29 mm (standard deviation: 44.746) for malignant MCN. Comparison of the size was performed by the Mann-Whitney-U-Test for continuous variables.*

Changes in the text: We added the data regarding the mean size of MCN in both populations to our manuscript on page 8, line 20 to page 9, line 2 for further clarification

Comment 8: How was exocrine sufficiency defined?

Reply 8: *Exocrine insufficiency was defined as preoperative stool elastase < 200 µg/g and/or clinical symptoms of malabsorption. Stool elastase tests are being routinely performed for all patients with pancreatic disease treated at our institution. Although the test has its limitations it offers a kind of quantitative measure and allows comparison between patients.*

Changes in the text: We added the definition to the “methods” section on page 6, line 19-20.

Comment 9: The authors note that there was a high portion of men (18.8%) in this study which is unusual given that 98% of MCNs are found in women. What is the explanation for such a high number? Can this study be generalizable?

Reply 9: *We absolutely agree with the reviewer. The unusually high proportion of males with MCN was somewhat disturbing to us too. However, this fact cannot be changed - all tissue samples were controlled by experienced pathologists, so we can only confirm the presented results.*

Changes in the text: We added a brief discussion of our findings on page 16, line 6 to 11.

Comment 10: The presence of symptoms such as abdominal pain and back pain, weight loss, jaundice diabetes, exocrine insufficiency, alcohol consumption, nicotine and ASA score were evaluated. Acute pancreatitis is an important factor to consider as many studies have looked at the presence of acute pancreatitis with malignancy and has been deemed as a worrisome symptom.

Reply 10: *In contrast to other malignant pancreatic entities, a relationship between acute pancreatitis and malignancy in MCN is not well established. Series of patients with MCN report a rate of 0 – 13 % regarding symptoms interpreted as a preoperative episode of acute pancreatitis. More than 50 % of the studies included in the systematic review by Nilsson et al in Pancreatology in 2016 did not report any patients with a history of acute pancreatitis. Of these studies, none found acute pancreatitis to be related to occurrence of MCN nor malignant transformation of MCN. In our study 11 (17.5%) patients had one or more episodes of acute pancreatitis before surgery. Two of them had malignant MCN and 9 had a benign MCN, 12.5% vs. 19%, not statistically*

significant. The relatively non-specific relation between pancreatitis and malignant potential of MCN may be due to the missing connection between pancreatic main duct and MCN.

Changes in the text: Analysis of history of acute pancreatitis as a risk factor for malignancy was added to table 3.

Comment 11: The authors found that 54% of patients with resected MCN had adenoma without dysplasia, and 25.4% have invasive carcinoma. The percentage of invasive carcinoma in this cohort is high compared to other studies.

Reply 11: *Yes, you are absolutely right! The high proportion of invasive carcinoma may be explained by a very strict positive selection of patients for surgery. Only those with really strong suspicion for malignancy underwent surgery, causing a high hit rate of malignant invasive cases in this series of consecutive patients.*

Changes in the text: We discussed this phenomenon in the text on page 15, line 20- page 16, line 2.

Comment 12: The authors looked at the tumor location and found that 33.4% were located in the head of the pancreas, which is also not common. However, there are other studies that have shown that MCN in the head of the pancreas may be associated with malignancy. Was this assessed in this study?

Reply 12: *We agree with the reviewer that MCN are reported to occur more frequently in the pancreatic tale. Regarding malignancy, our literature search revealed only one article demonstrating a significant connection between a tumor location in the pancreatic head and malignancy (Zamboni et al, The American Journal of Surgical Pathology, 1999). This finding was not replicated by later studies (e. g. Keane et al, British Journal of Surgery, 2017). There are however numerous studies demonstrating a more frequent occurrence of pancreatic head MCN in male patients. We also found a strong association between tumor location in the pancreatic head and male sex ($p > 0.001$).*

Changes in the text: We added analysis of the tumor location as a potential categorical risk factor for malignancy to table 3. We also added our findings regarding tumor location and sex on page 8, line 18-22 and added a brief discussion regarding the subject on page 14, line 23 – page 15, line 2.

Comment 13: The authors have included 4 tables. Table 1 is demographic table addressing the characteristics, clinical, radiologic, surgical and post op findings of the cohort. Table 2 shows comparison of continuous parameters and table 3 shows comparison of categorical parameters. Table 4 shows the logistic regression analysis. Consider adding tumor location as a parameter. Consider ROC curve for continuous variables. Consider addition of Kaplan Meier curve.

Reply 13: *We agree that a ROC curve could improve understanding of the relationship between the continuous preoperative variables and malignancy in MCN. We added a ROC curve regarding the relationship between CEA and CA19-9 levels with malignancy as these parameters showed a low p value in the Mann-Whitney-U-test.*

Changes in the text: We added Figure 3, presenting the ROC curves of CEA and CA19-9 levels. We also added the results of our AUC analysis on page 8, line 7-11.

We also added results of the Fisher's Exact test comparing elevated CEA and CA19-9 levels to table 3. These findings were previously only described in the result section of our manuscript.

Kaplan-Meier curves were also included as already discussed in comment 3.

Comment 14: The study is limited by its retrospective design and the inclusion of patients that had resected MCN, thereby producing selection bias. The latter should also be addressed as a limitation of the study.

Reply 14: *This is correct.*

Changes in the text: We modified the discussion of our study on page 19, line 5-7 to emphasize the selection bias caused by the study design.