

To the reviewers:

We sincerely thank the peer reviewers for their time and thoughtful comments in consideration of our manuscript: Clinical outcomes of ampullary neoplasms in resected margin positive or uncertain cases after endoscopic papillectomy. We have addressed their comments, and upgraded and improved the text accordingly. We feel the updated manuscript is more clear and comprehensive. Please see below for responses point to point. (Reviewers comments below in bold).

Reviewer 1 (#03475728, Italy)

We really enjoyed the opportunity to review the manuscript titled, "Clinical outcomes of ampullary neoplasms in resected margin positive or uncertain cases after endoscopic papillectomy". The article focuses on a interesting and controversial topic: the endoscopic management of ampullary tumors. Despite it's retrospective character, it is an interesting and timely study. I would suggest to bring some minor revisions, which I will try to list below:

1.Considering such a hot topic, widely debated in the literature, I would enrich the reference list, discussing some interesting papers that have already faced this theme, e.g.: •Ardengh et al." Endoscopic papillectomy: The limits of the indication, technique and results". World J Gastrointest Endosc. 2015;7(10):987-94. •De Palma et al. "Endoscopic snare papillectomy: a single institutional experience of a standardized technique. A retrospective cohort study" Int J Surg. 2015. • Nam K. et al. "Usefulness of argon plasma coagulation ablation subsequent to endoscopic snare papillectomy for ampullary adenoma" Dig Endosc. 2018 •Riditid Wet al. "Endoscopic papillectomy: risk factors for incomplete resection and recurrence during long-term follow-up". Gastrointest Endosc. 2013;79(2):289-96.

Thank you very much for your comments. We added some sentences and these references in revised manuscript.

Page 7, Line2 ([2] De Palma GD, et al.)

Endoscopic papillectomy (EP) for benign ampullary neoplasms could be a less-invasive alternative to pancreatoduodenectomy (PD) because PD might be an excessively invasive treatment for benign neoplasms [1,2].

Page 7, Line12 ([5] Ardengh JC, et al.)

Most episodes of bleeding can be controlled by endoscopic hemostasis and most episodes of pancreatitis are mild and resolved with conservative treatment [5].

Page14, Line24 ([21] Ridditid W, et al.)

In the literature of Ridditid W, et al., among patients with ampullary adenoma who had complete resection (n=107), 16 patients (15%) developed recurrence up to 65 months after resection [21].

Page15, Line12 ([22] Nam K, et al.)

On the other hand, Nam K et al reported usefulness of APC subsequent to EP [22]. In this literature, they concluded additional APC during EP may have a beneficial effect by decreasing bleeding rate without harmful effects, but not have an effect of decreasing tumor recurrence.

2. Pancreatoduodenectomy is considered to be too invasive for benign neoplasms, so endoscopic papillectomy is proposed as an alternative: the differences in complications, morbidity/mortality, between the two procedures should probably be better emphasized.

Thank you for your suggestion. We have reflected this comment in 'Introduction' section.

Page 7, Line 5

The current standard treatment for ampullary tumor is PD, which is still associated with a high morbidity rate despite a recent reduction in the mortality rate to less than 5% [3, 4]. On the other hand, the mortality after EP has been reported by high volume centers to be 0.4%. The overall complication rate of EP reported between 8% and 35%, and the most common complications are pancreatitis (5%-15%) and bleeding (2%-16%). Most episodes of bleeding can be controlled by endoscopic hemostasis and most episodes of pancreatitis are mild and resolved with conservative treatment. [5].

3. You assert that “main” indications for EP was adenoma. Do you use other selection criteria, e.g. the lesion size? ... this should be discussed.

In the cases with the evidence of ampullary carcinoma by the preoperative biopsy, basically, PD was recommended. But, in some cases, such as super-elderly patients or patients in a poor general condition, EP was performed after obtaining informed consent. These comments were described in 'MATERIALS AND METHODS (Patients)' section.

Page 9, Line 5

In the cases with evidence of ampullary carcinoma by the preoperative biopsy, PD was recommended. In some cases, such as those involving super-elderly patients or patients in a poor general condition, EP was performed when T2 invasion was not detected by preoperative imaging studies.

4. In the RESULTS section, the authors say that 12 lesions were diagnosed as adenocarcinoma; follow-up was selected for 5 adenocarcinoma, while additional PD was performed in 4 cases: what about the other 3 adenocarcinoma?

Thank you for your comment. In 12 adenocarcinomas, 9 cases were resected margin-uncertain/positive, and 5 of them were selected follow-up and 4 were PD. Remaining 3 cases were resected margin-negative cases. Please see Figure 5.

5. With regard to technical details, the authors say that they “tried to insert both bile duct and pancreatic stent after the EP procedure”; it might be interesting to discuss this attitude and current evidence for it. More, regarding the resection technique, it would be interesting to understand if they tried to attempt an en block resection in all the cases and, eventually, which were the reasons for failure.

Thank you for your comment. We added some sentences and references in 'DISCUSSION' section.

Page 15, Line 17

Pancreatitis is the most common problematic complication of EP. Currently, several studies have shown that placement of a prophylactic pancreatic stent after EP reduces the risk of pancreatitis. A prospective, randomized, controlled trial showed that pancreatic duct stenting after EP significantly decreased the rate of post-EP pancreatitis [23]. However, whether or not post EP pancreatic stenting can alleviate the rate of pancreatitis is still controversial. Some studies have shown no statistically significant benefit by prophylactic placement of a pancreatic stent during EP. In this study, post-EP

pancreatitis occurred in 13 patients (28.9%). Prophylactic pancreatic stent had been placed in all post-EP pancreatitis patients. Further prospective controlled studies would be required to evaluate the efficacy of prophylactic pancreatic stenting after EP for prevention of post-EP pancreatitis.

6.Regarding the follow-up protocol, it would be nice to discuss a bit if there are any current established recommendation.

Thank you for your important suggestion. Unfortunately, established recommendation of follow-up protocol after EP has not been published. This point should be addressed in the future investigation. These comments were described in 'DISCUSSION' section.

Page 14, Line 30

But, established recommendation of follow-up protocol after EP has not been published. This point should be addressed in the future investigation.

7. Some evidence from literature regarding the role of APC after EP should be probably discussed.

There is no consistent evidence of APC after EP. There were some reports which described the usefulness of APC for recurrent tumor after EP. On the other hand, additional APC during EP may have a beneficial effect by decreasing bleeding rate without harmful effects, but not have an effect of decreasing tumor recurrence. Some sentences were added in 'DISCUSSION' section.

Page 15, Line 6

APC has been reported to be useful for recurrent ampullary tumors that arise after EP [1,9,15,17].

Page 15, Line 12

On the other hand, Nam K et al reported usefulness of APC subsequent to EP [22]. In this literature, they concluded additional APC during EP might have a beneficial effect by decreasing bleeding rate without harmful effects, but not have an effect of decreasing tumor recurrence.

8.The retrospective nature of the study along with the small sample size should be emphasized before concluding that positive or uncertain resected margin

could be managed by APC. Prospective and comparative data are probably required.

We totally agree with your opinion. That is described in 'DISCUSSION' section.

Page 16, Line 4

Second, the sample size was too small to allow the results to be generalized. A further large-scale prospective study will be needed in future.

Reviewer 2(#03476031,Sweden)

Nice retrospective study but with only 45 cases over almost 12 years so quite a small case load to draw conclusions from and no really new conclusions are drawn except that the utility of determining radical resection is probably of no use. The study aims at determining the clinical outcome of patients that has gone thru EP were the resected specimen has undetermined or positive resection margins. They show that the outcome is probably the same as if the margins were negative, at least for adenomas with recurrence 1/18 or 2/15. Adenocarcinomas were found in both study groups and the authors do not present in what degree the preinterventional biopsies showed adenocarcinoma or adenoma. EP is an established treatment method for the treatment of adenomas in the major duodenal papilla. One question that still remains is at what level of adenocarcinoma invasion is it safe to treat these patients. And how to stage this correctly before any resection is made? No prospective study has to my knowledge been made where patients with biopsy proven adenocarcinoma and thorough staging is allocated to either EP or PD. Instead conclusions regarding the resectability of early adenocarcinomas (Tis or T1a) is made from retrospective studies. The feasibility and results regarding adenoma resection have been shown several times previously in several papers. To make the paper a bit more interesting I would prefer that more data regarding the preinterventional investigations were included.

- What did the preinterventional biopsies show?
- How many had adenoma or adenocarcinoma already before the EP?
- How did that correlate with the results from the resected specimen?

Thank you very much for your comments. We added the new Table 2, and described 'RESULTS (Baseline characteristics of this study)' section.

Page 11, Line 11

The diagnostic accuracy of endoscopic forceps biopsy before EP was 68.9% (31/45), with underestimated diagnosis in 2.2% (1/45) (Table 2).

- **Estimated size of the lesions before the resections?**
- **What clinical signs did the patients have? Jaundice? Abdominal pain?**

Thank you for your valuable comments. We added this information in Table 1. Please see revised Table 1. In all cases, there is no symptom such as jaundice and abdominal pain.

- **Define more precisely a super-elderly? How many were they?**

There were 6 super-elderly (over 80 years old) in this study. We have reflected this comment in 'RESULTS (Baseline characteristics of this study)' section.

Page 11, Line 5

6 patients were over 80 years old (13.3%).

- **What was the performing endoscopists impression regarding the radicality of the resection? Did they believe that the resection was complete or not?**

In all cases, endoscopists believe that the resection was complete, even though it became piece meal resection. We have added this point in 'MATERIALS and METHODS (EP procedure and evaluation of resected specimens)' section.

Page 9, Line 23

Even though it became piecemeal resection because of its size, the tumor was endoscopically completely resected in all cases.

- **Were all patients examined with EUS and IDUS? At what level of invasion did they have a cutoff to deem them irresectable with EP?**

EUS were performed in all patients. IDUS was attempted whenever feasible. In the cases with evidence of ampullary carcinoma by the preoperative biopsy, PD was recommended. In some cases, such as those involving super-elderly patients or patients in a poor general condition, EP was performed when T2 invasion was not

detected by preoperative imaging studies. These sentences were added in 'MATERIALS AND METHODS (Patients)' section.

Page 9, Line 5

In the cases with evidence of ampullary carcinoma by the preoperative biopsy, PD was recommended. In some cases, such as those involving super-elderly patients or patients in a poor general condition, EP was performed when T2 invasion was not detected by preoperative imaging studies.

• **Were there no changes in their approach to these patients over the 12 years?**

Our approach for benign ampullary neoplasms has not been changed over the 12 years. However, recently, in some cases, such as those involving super-elderly patients or patients in a poor general condition, EP was performed when T2 invasion was not detected by preoperative imaging studies. However, there is no consistent evidence on the validity of EP for malignant ampullary tumors. We also think it would be current important issue on considering the appropriate indication of EP.

• **How did they define their complications? I would also like them to discuss and hopefully present at what rate they succeed with pancreatic stenting and if that correlated to the relatively high rate of post interventional pancreatitis.**

Post-EP bleeding was defined as hematemesis and/or melena or a >2 g/dL decrease in hemoglobin level (bleeding requiring hemostasis). Immediate bleeding was not defined as post-EP bleeding. Post-EP pancreatitis was defined as an increase in serum amylase/lipase to >3 times the upper limit of normal, along with typical pancreatic-type pain. Prophylactic both pancreatic and biliary stent was placed in 33 (73.3%) patients, only pancreatic stent was placed in 7 (15.6%) patients, and only biliary stent was placed in 4 (8.9%) patients; there was no stent placement in 1 (2.2%) cases. Prophylactic pancreatic stent was placed in all post-EP pancreatitis patients. Pancreatic stenting did not correlate with the occurrence of post-EP pancreatitis. These sentences were added in 'RESULTS (Baseline characteristics of this study)' section.

Page 11, Line8

Prophylactic both pancreatic and biliary stent was placed in 33 (73.3%) patients, only pancreatic stent was placed in 7 (15.6%) patients, and only biliary stent was placed in 4 (8.9%) patients, there was no stent placement in 1 (2.2%) cases. Complications

occurred in 42.2% of all patients. Hemorrhage occurred in 9 patients (20.0%), and pancreatitis occurred in 13 patients (28.9%). 1 of 9 post-EP hemorrhage cases was severe bleeding which was needed TAE (transcatheter arterial embolization). 1 of 13 post-EP pancreatitis cases was severe acute pancreatitis. Prophylactic pancreatic stent was placed in all post-EP pancreatitis patients. There were no complication related deaths.

• One of the few conclusions that can be made from this study is that the issue of negative resection margins or not is probably of little interest. Instead, that a strict follow-up regimen is applied to find any signs of recurrence and the bit question still remains, if one dares (or finds it ethically appropriate) to randomize between EP or PD in patients with biopsy proven adenocarcinoma and a strict preinterventional staging.

Thank you for your comments. Your comment is important and we totally agree with you. We also think randomized investigation between EP and PD has ethical problem, because there is no consistent evidence of the prognosis after EP for malignant tumor. We also hope this issue would be resolved in future.

Reviewer 3(# 00069105, Spain)

Dear sirs: You have great experience in the endoscopic treatment of ampullar tumors. Comments.

Introduction: you do not mention surgical ampullectomy that is an alternative between endoscopic treatment and PD

Thank you for your suggestion. We have reflected this comment in 'Introduction' section.

Page7, Line14

Surgical ampullectomy (SA) has also existed as an alternative to PD. Ceppa et al showed that EP was found to have equivalent efficacy when compared with SA. Moreover, EP had lower morbidity and identical mortality [6].

Material and Methods. IDUS explanation should be improved. What do you mean if it is feasible.

To diagnose intraductal involvement, EUS was performed for all EP cases. IDUS was attempted for cases which were difficult to diagnose intraductal involvement by EUS, because of the risk of pancreatitis. These sentences were added to 'MATERIALS AND METHODS (Patients)' section.

Page 8, Line 31

IDUS was attempted for cases which were difficult to diagnose intraductal involvement by EUS, because of the risk of pancreatitis.

How many patients biopsy was positive for adenocarcinoma but they were unfit for surgery. Exclusion criteria for surgery was age, Charlson Index,... why surgery was not done after relapse?

Preoperative biopsy diagnosed as adenocarcinoma was only 4 patients in this study. In these cases, they were unfit for surgery because 1 patient was super-elderly (85 years old), and 3 patients were poor general condition with severe comorbidity. These sentences were added in 'MATERIALS AND METHODS (Patients)' section.

Page 9, Line 9

Preoperative biopsy diagnosed as adenocarcinoma was only 4 patients in this study. In these cases, they were unfit for surgery because 1 patient was super-elderly (85 years old), and 3 patients were poor general condition with severe comorbidity.

Hemorrhage and pancreatitis were severe/mild? mortality? need for surgery or endoscopy treatment due to complications?

1 of 9 post-EP hemorrhage cases was severe bleeding which needed TAE. 1 of 13 post-EP pancreatitis cases was severe acute pancreatitis. There were no complication related deaths. These sentences were added in 'RESULTS (Baseline characteristics of this study)' section.

Page 11, Line 14

1 of 9 post-EP hemorrhage cases was severe bleeding which was needed TAE (transcatheter arterial embolization). 1 of 13 post-EP pancreatitis cases was severe acute pancreatitis. Prophylactic pancreatic stent was placed in all post-EP pancreatitis patients. There were no complication related deaths.

The decision of follow up or PD after positive margin on adenocarcinoma was based ???

Thank you for your important suggestion. That has been described in 'MATERIALS AND METHODS (EP procedure and evaluation of resected specimens)' section.

Page10, Line10

After examining the resected specimens, additional PD was recommended in the cases of adenocarcinoma. However, in the cases in which additional PD could not be performed based on the patient's wishes or condition, we chose to perform palliative endoscopic treatment or medical follow-up.

Mean follow up and free disease and total survival 1 year 2 year and 5 year of ampullectomy done in malignant tumors?

In this study, no ampullary tumor-related deaths occurred in all patients. Median observation period was 27.1 months (range: 3.0-133.4). That has been described in 'RESULTS (Baseline characteristics of this study)' section.

Page11, Line26

The median duration of the follow-up period was 27.1 months (range: 3.0–133.4).

Hospital stay?

The mean hospital stay was 15.4 days. We have added this point in 'RESULTS (Baseline characteristics of this study)' section, and revised Table1.

Page11, Line31

The mean hospital stay was 15.4 days.

Reviewer 4 (#03388124, United States)

This is a very nice study that evaluated the clinical outcome of endoscopically resected ampullary tumors with positive/equivocal margins. It has potential implications for patient management and prognostication. This manuscript is well-written and appears acceptable for publication.

1. Recommend to include statistical analysis (e.g. Fisher's exact test) between adenoma and adenocarcinoma to strengthen the manuscript.

Thank you for your valuable comment. In 20 follow-up cases of resected margin positive or uncertain cases, local recurrence was detected in 3 cases. In 21 resected margin negative cases, local recurrence was detected in 1 case. There was no significant difference in the local recurrence between resected margin positive/uncertain and negative cases ($p=0.34$, 2-sided Fisher's exact test). In the local recurrent cases, 3 of the primary lesions were diagnosed as adenomas after EP, and 1 was diagnosed as an adenocarcinoma. There was no significant difference in the local recurrence between adenomas and adenocarcinomas ($p=0.99$, 2-sided Fisher's exact test). We have added these sentences in 'RESULT (Details of the cases involving post-EP recurrence)' section.

Page 13, Line 4

In 20 follow-up cases of resected margin positive or uncertain cases, local recurrence was detected in 3 cases. In 21 resected margin negative cases, local recurrence was detected in 1 case. There was no significant difference in the local recurrence between resected margin positive/uncertain and negative cases ($p=0.34$, 2-sided Fisher's exact test). In the local recurrent cases, 3 of the primary lesions were diagnosed as adenomas after EP, and 1 was diagnosed as an adenocarcinoma. There was no significant difference in the local recurrence between adenomas and adenocarcinomas ($p=0.99$, 2-sided Fisher's exact test).

2. Recommend to include representative microscopic histopathologic images (adenoma and adenocarcinoma with positive/equivocal margins).

Thank you for your suggestion. We added new Figure 3.

3. For the follow-up period, recommend to use "months" instead of "days".

Thank you for your suggestion. We have changed the manuscript to use months instead of days, and revised table1 and table3.

4. Recommend to spell out the full word for "PD" at first appearance in the abstract.

Thank you for your suggestion. We have reflected this comment in 'Abstract' section.

Page4, Line3

Endoscopic papillectomy (EP) for benign ampullary neoplasms could be a less-invasive alternative to pancreatoduodenectomy (PD).

5. Is there any difference between a positive HM and positive VM?

Thank you for your comment. We have added this sentence in 'RESULT (Details of the cases involving post-EP recurrence)' section.

Page13, Line 18

There is no difference in the post-EP recurrence between the positive or uncertain HM and positive or uncertain VM.

6. Based on your study, would you recommend endoscopic therapy only for T1a or T1b adenocarcinoma?

Thank you for your valuable comment. In this study, we concluded that resected margin positive or uncertain cases after EP could be managed by endoscopic treatment including APC, even in cases of adenocarcinoma. But it's difficult to refer the indication of EP for adenocarcinoma because of small sample size.

7. During the study time period, have you treated any neuroendocrine tumors with endoscopic resection? Any comments on the clinical outcome of those tumors with positive margins, compared to adenoma/adenocarcinoma?

You have raised an important point. However, we have no neuroendocrine tumors in this study period.