

Dear editors and reviewers,

On behalf of my co-authors, we thank you very much for allowing us to revise our manuscript. We appreciated the editors and reviewers very much for their positive and constructive comments and suggestions on our manuscript. These comments are all valuable and helpful for revising and improving our paper, as well as the important guiding significance to our study. We have studied the comments carefully and have made corrections *in red* in the manuscript, which we hope to meet with approval.

Before we give our response to the science editor and reviewers's comments, we ask for permission from the editors to change the paper's title based on Reviewer 1's suggestion to be:

ERCP in elderly patients; difficult cannulation and adverse events

The responses to the comments of Science Editor Le Zhang are as follows:

Thank you for your valuable comments on the formatting of the manuscript.

We have modified the manuscript based on the format file that you sent to us. The figures and tables are moved to the end of the manuscript. Also, we added the manuscript highlights at the end of the manuscript.

The responses to the reviewer's comments are as follows:

Reviewer #1

Comments to the Author

- 1) The methodology is neither clear nor uniform. Specific questions with regard to cannulation are as follows:
 - a) What was the technique used initially? Is it sphincterotome with guidewire or cannula with guidewire.
 - b) If the initial attempts fail does the endoscopist go to needle knife or pancreatic sphincterotomy, in any particular order uniformly in every patient or does the endoscopist pick and choose the technique based on his expertise.

Response:

Thank you for your valuable comments. We have rewritten the whole materials and method section to make it more clear and uniform based on your suggestions. Here we give the answers to the two points that you asked:

- a) The initial technique was guidewire-assisted technique cannulation with sphincterotome.
- b) Choosing the suitable secondary cannulation technique was according to the expertise of endoscopists, which was mainly based on the repetitive unintended guidewire insertion into the pancreatic duct. Needle-knife precut (NKP) was considered as the preferred choice in the absence of pancreatic cannulation and also the cases with impacted biliary stones at the ampulla or the distal CBD. NKP was performed with a needle-knife sphincterotome. In the cases of prior pancreatic guidewire insertion, Transpancreatic sphincterotome (TPS) was performed with placement of a prophylactic pancreatic stent.

We have mentioned these details in the methods section based on your suggestion.

Comments to the Author

- 2) Almost 1/3rd of the patient underwent ERCP had difficult cannulation. This seems to be much higher than the published data.

Response:

Thank you for your valuable comment. The published data varies according to the center type and the used criteria to identify the difficult cannulation. Our center is a tertiary center that receives

complicated referral cases from other centers. The definition of difficult biliary cannulation is highly variable among studies and there is no uniform definition of cannulation attempt. Some studies in the literature have a comparable difficult cannulation ratio to our study. For example, in [1], the primary cannulation success rate was 74.9% which means the difficult cannulation ratio was (25.1%); in [2], the difficult cannulation ratio was (32.4%).

References:

[1] Halttunen J, Meisner S, Aabakken L, Arnelo U, Grönroos J, Hauge T, Kleveland PM, Nordblad Schmidt P, Saarela A, Swahn F, Toth E, Mustonen H, Löhr JM. Difficult cannulation as defined by a prospective study of the Scandinavian Association for Digestive Endoscopy (SADE) in 907 ERCPs. *Scandinavian Journal of Gastroenterology* [Internet] 2014;49:752–8 [PMID: 24628493 DOI: 10.3109/00365521.2014.894120]

[2] Ukkonen M, Siiki A, Antila A, Tyrväinen T, Sand J, Laukkarinen J. Safety and Efficacy of Acute Endoscopic Retrograde Cholangiopancreatography in the Elderly. *Digestive Diseases and Sciences* [Internet] 2016;61:3302–8 [PMID: 27565508 DOI: 10.1007/s10620-016-4283-2]

Comments to the Author

3) The number of patients in the difficult cannulation group over 80 yrs of age is very small compared to those who are under 80 yrs of age. Can this affect the statistics?

Response:

Thank you for your valuable comment. Our original sample size was reasonable as it is a single-center study. However, the analyses were limited by the small size of the elderly population. To overcome this issue, we performed Fisher's exact test (which is more accurate with a small sample size) to determine the differences among different patient cohorts.

Comments to the Author

4) The total number in table 2 and the numbers shown for group A and group B do not add up to be equal.

Response:

Thank you for your valuable comment. We followed your comment and corrected the calculation's mistakes in Table 2, and as a result Table 3 is revised.

Comments to the Author

5) Minor points: 1. Change the title to ERCP in elderly patients; difficult cannulation and adverse events. 2. Page 3 para 2: Delete the first two sentences and start with although multiple studies..... 3. Page 4 para 3: Sentence 7 - change 'applied' to 'deployed'

Response:

Thank you for your kind suggestions. We changed the title of the manuscript based on your suggestion. Also, we modified the text based on your suggested points.

The responses to the reviewer's comments are as follows:

Reviewer #2

Comments to the Author

- 1) Authors should include in the introduction more detailed information on the criteria used to classify cannulation as difficult (number of cannulation attempts and time to cannulation)

Response:

Thank you for your valuable comment. We have included the required information on the classification of difficult cannulation in the introduction.

Comments to the Author

- 2) CCI score criteria should be included in the methods in order to make it clearer to the reader.

Response:

Thank you for your valuable comment. We followed your suggestion and added the CCI score criteria in the methods section.

Comments to the Author

- 3) Authors should comment whether they used any criteria to identify the severity of post-ERCP pancreatitis.

Response:

Thank you for your valuable comment. We added a sentence to clarify the severity of PEP in our sample in the results section as follows:

PEP was the most frequent adverse event in both groups with a lower incidence in the elderly patients' group without a significant difference (2.7% vs. 6%, $p=0.088$). Most patients in both groups were diagnosed with mild pancreatitis; only two cases of moderate pancreatitis developed in the younger group, and all patients were treated conservatively.

Comments to the Author

- 4) Authors should comment in methods if any sample size calculation was used on the study.

Response:

Thank you for your valuable comment. We followed your comment and added in the methods section the information on how the sample size of the study is determined as follows:

Our sample size was larger than the theoretical sample size estimated by (Gpower 3.1, α 0.05; statistical power 80%; 1:3 allocation ratio, Fisher's exact test) and can lead to sufficient conclusions.

The responses to the reviewer's comments are as follows:

Reviewer #3

Comments to the Author

1) Several abbreviations should be spelled out in the main text (ERCP, PEP, CCI,...).

Response:

Thank you for your valuable comment. We have followed your comment and spelled out the mentioned abbreviations in the main text.

Comments to the Author

2) How did authors determine the sample size of this study?

Response:

Thank you for your valuable comment. For the statistical selection of the sample size in our study: we followed the statistical formulas of sample sizes for two independent samples, dichotomous outcomes. First, the allocation ratio of the two groups is selected as (1:3). The statistical power is chosen as 80%, which is sufficient to get reasonable conclusions. The selected level of significance is 0.05. The statistical total sample size is 571 (142 in the elderly group, 429 in the younger group). The actual power is 0.80, and the actual level of the significance is 0.042. In our study, we enrolled more than the theoretical sample size 614 (146 in the elderly group, 468 in the younger group).

We added this sentence in the methods section to clarify the sample size determination:

Our sample size was larger than the theoretical sample size estimated by (Gpower 3.1, α 0.05; statistical power 80%; 1:3 allocation ratio, Fisher's exact test) and can lead to sufficient conclusions.

Comments to the Author

3) What is the anatomy of the papilla? Authors should explain it.

Response:

Thank you for your valuable comment. Size of the papilla including small and protruding papilla, in addition to other variant anatomy in

the orientation and location of the papilla were reported under the cause of papillary anatomy. We have added this explanation in the methods section based on your suggestion.

Comments to the Author

- 4) The name of group is difficult to understand. Would you please change the name of group (for example, Younger group, or Older group)?

Response:

Thank you for your valuable comment. We followed your suggestion and changed the groups' names to be more clear.

Comments to the Author

- 5) Figure 1 is difficult to understand. Patients who were difficult to cannulate should be set below the "Based on age"

Response:

Thank you for your valuable comment. You are right. We corrected the figure based on your suggestion.

Comments to the Author

- 6) The "RESULTS" section is wordy. The authors should describe compactly.

Response:

Thank you for your valuable comment. We followed your suggestion and rephrased the results section to be clear and compact.

Comments to the Author

- 7) Is the Table 3 necessary? This is the comparison between three groups. The authors should perform sub effect tests.

Response:

Thank you for your valuable comment. In Table 3, we aimed to study the safety of the cannulation techniques in older and younger groups. You are right that we had to perform sub-effect tests to check the difference between every two techniques in case of a significant difference is obtained. The only PEP in younger group patients had a significant difference between the three techniques. Since our paper focusses on the elderly population and based on your comment, if the table is necessary, we deleted the rows that are related to the younger group from Table 3. As a result, Table 3 shows that there is no significant difference in the incidence of overall adverse events and particularly PEP when using different cannulation techniques. So, there is no need to continue the sub-effect tests for the elderly group

patients. We modified Table 3 and its corresponding explanation in the text.

Comments to the Author

8) What is the definitions of Charlson score, Second ERCP, papillary morphology, CCI? The authors should explain the examination items in the “METHOD” part.

Response:

Thank you for your valuable comment. We followed your suggestion and defined the terms mentioned above in the methods section. For the papillary morphology, we replaced it with the term “papillary anatomy” which was explained in the methods section to be more clear.

Comments to the Author

9) There were few patients who got PEP to perform the multivariate analysis.

Response:

Thank you for your valuable comment. First, considering the rarity of PEP cases, to get more PEP cases, we need a large sample size of ERCs in the study. As our study is a single-center prospective study, our focus was to give an overview of the PEP’s risk factors. On the other hand, in the literature, several works performed the multivariate analysis for risk factors of a small number of adverse events cases. For example, [3] had 16 PEP cases and 4 independent variables in their multivariate analysis; [4] had 16 overall adverse events cases and 2 variables in their multivariate analysis; [5] had 32 PEP cases and 7 variables in their multivariate analysis. Based on your comment, we added a note to the limitations paragraph of this study as follows:

There are a few limitations to this study. First, the study was performed at a single center with a limited number of elderly patients. The second limitation is the rarity of specific adverse events, especially PEP, which may affect the results of multivariate analyses.

References:

[3] Lee YK, Yang MJ, Kim SS, Noh C-K, Cho HJ, Lim SG, Hwang JC, Yoo BM, Kim JH. Prediction of Post-Endoscopic Retrograde Cholangiopancreatography Pancreatitis Using 4-Hour Post-Endoscopic Retrograde Cholangiopancreatography

Serum Amylase and Lipase Levels. Journal of Korean Medical Science [Internet] 2017;32:1814 [DOI: 10.3346/jkms.2017.32.11.1814]

[4] Ukkonen M, Siiki A, Antila A, Tyrväinen T, Sand J, Laukkarinen J. Safety and Efficacy of Acute Endoscopic Retrograde Cholangiopancreatography in the Elderly. Digestive Diseases and Sciences [Internet] 2016;61:3302–8 [PMID: 27565508 DOI: 10.1007/s10620-016-4283-2]

[5] Iorgulescu A, Sandu I, Turcu F, Iordache N. Post-ERCP acute pancreatitis and its risk factors. Journal of medicine and life [Internet] 2013;6:109–13 [PMID: 23599832]

Comments to the Author

10) The DISCUSSION section is lengthy. You should describe about the items that was statistically different between two groups.

Response:

Thank you for your valuable comment. We followed your suggestion and modified the discussion section and deleted the parts related to items that are not statistically different.

The responses to the reviewer's comments are as follows:

Reviewer #4

Comments to the Author

This article is significant as a prospective study; however, this is not a new topic. You should investigate 90 years or older. Further, please indicate the statistically required sample size.

Response:

Thank you for your good comments. We respect your evaluation of our manuscript. We know that in the ERCP literature, there are several studies focused on the safety and efficacy of ERCP procedure and its outcomes in elderly patients. However, there is a lack of studies focused on the difficult cannulation in the elderly population. This is the main aim of our manuscript, which is different from other studies in the literature. For your suggestion to perform the study for patients with 90 years or older, we also agree with you that it will be more interesting, but at a single center, it needs much longer time to enroll patients from this population.

For the statistical selection of the sample size in our study: we followed the statistical formulas of sample sizes for two independent samples, dichotomous outcomes. First, the allocation ratio of the two groups is selected as (1:3). The statistical power is chosen as 80%, which is sufficient to get reasonable conclusions. The selected level of significance is 0.05. The statistical total sample size is 571 (142 in the elderly group, 429 in the younger group). The actual power is 0.80, and the actual level of the significance is 0.042. In our study, we enrolled more than the theoretical sample size 614 (146 in the elderly group, 468 in the younger group).

We added this sentence in the methods section to clarify the sample size determination:

Our sample size was larger than the theoretical sample size estimated by (Gpower 3.1, α 0.05; statistical power 80%; 1:3 allocation ratio, Fisher's exact test) and can lead to sufficient conclusions.