Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Association of proton pump inhibitors with the risk of hepatic encephalopathy in advanced liver disease: a meta-analysis" (ID: 47009). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. The responds to the reviewers' comments are as following:

Reviewer #1:

We thank Reviewer #1 for the constructive and insightful criticisms and suggestions. Below is our point-by-point response to the reviewer's comments.

① **Reviewer's comment:** "different type of study are considered. In particular there are conference abstracts. These studies, generally, have no definitive conclusions and in this case could provide misleading results. I suggest to eliminate abstract from the analisys, being result slight similar."

Response:

We agree with the reviewer that there is some uncertainty in the conclusions of conference abstracts. However, according to the Cochrane Collaboration Handbook V 5.1.0(Part 2: General methods for Cochrane reviews > 6 Searching for studies), finding out about unpublished studies, and including them in a systematic review when eligible and appropriate, is important for minimizing bias. Conference abstracts and other grey literature can be an important source of studies for inclusion in reviews[1]. In addition, Many meta-analyses included and analyzed data from conference abstracts[2-4]. In the same way as their analyses, we also performed the sensitivity analysis by excluding the data from conference abstracts, as the reviewer commented, the result was almost unchanged**(See Figure 3;See revised manuscript Page 11, Line302-304; Page 12, Line328-329).** We feel that it would be beneficial to include conference abstracts, in order to minimize selection bias and publication bias.

Reference:

1. Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from http://handbook.cochrane.org.
2. Polachek A, Touma Z, Anderson M, et al. Risk of cardiovascular morbidity in patients with psoriatic arthritis: a meta-analysis of observational studies[J]. Arthritis care & research, 2017, 69(1): 67-74.
3. Hagan H, Jordan A E, Neurer J, et al. Incidence of sexually-transmitted hepatitis C virus infection in HIV-positive men who have sex with men: A systematic review and meta-analysis[J]. AIDS, 2015, 29(17): 2335.
4. Persson M S M, Sarmanova A, Doherty M, et al. Conventional and biologic disease-modifying anti-rheumatic drugs for osteoarthritis: a meta-analysis of randomized controlled trials[J]. Rheumatology, 2018, 57(10): 1830-1837.

② **Reviewer's comment:** "The study by Lin et al is aimed to assess the role of PPI in a

particular setting: the acute HBV infection on chronic liver disease. This is, maybe, the case of an Acute HE and not the C-type HE. Please explain As well known HE is frequently consequent a precipitating event. Please calarify and add results following this suggestion"

Response:

We thank the reviewer for pointing this out, and the comments are very helpful for us to improve our paper. Hepatic encephalopathy can be subdivided in type A, B and C depending on the underlying cause. Type A describes hepatic encephalopathy associated with acute liver failure(ALF), type B is caused by portal-systemic shunting without associated intrinsic liver disease, and type C occurs in people with cirrhosis[5]. Acute liver failure (ALF) is a syndrome of diverse etiology, in which patients without previously recognized liver disease[6]. Unlike ALF, acute-on-chronic liver failure(ACLF) combines an acute deterioration in liver function in an individual with pre-existing chronic liver disease(cirrhosis and chronic but non-cirrhotic liver disease)[7]. The study by Lin et al [8] chose hepatitis B virus-related acute-on-chronic liver failure as study subject, the proportion of patients with cirrhosis was over 50% (See the Results section "50.9%vs. 51.8%"). Therefore, most patients included by Lin et al may be considered C-type HE. As the reviewer commented, the study by Lin et al was aimed to assess the role of PPI in a particular setting. To avoid the impact of this type of liver disease on the results, we perform a specific sensitivity analysis by excluding Lin's study, the pooled RR is slightly altered (from 2.14 [1.54, 2.97] to 1.99 [1.44, 2.74]). This shows that our conclusion is robust. In our meta-analysis, the search terms only included two parts: PPI and hepatic encephalopathy. We retrieved all studies that evaluated the association between PPI and HE regardless of the type of liver disease. We did not find other studies similar to Lin's study, allowing us to conduct further analysis for this particular setting.

We have added the following discussions on these suggestions in our revised manuscript.

See revised manuscript Page 13, Line352-354

"the study by Lin et al was aimed to assess the role of PPI in a particular setting(ACLF), sensitivity analysis by excluding the study showed the pooled risk estimate was slightly altered."

See revised manuscript Page 15, Line400-406

"HE in patients with ACLF seems to be different from that of acute decompensation in the clinical and pathophysiological aspect, the mechanism and classification are still unknown. Systemic inflammation, impaired intestinal mucosal immunity and changes of intestinal microbiota may increase the risk of bacterial translocation in patients with ACLF. Using PPI in ACLF patients appears to further increase the risk of HE, which requires further research."

Reference:

5. Ferenci P, Lockwood A, Mullen K, et al. Hepatic encephalopathy—definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998[J]. Hepatology, 2002, 35(3): 716-721.
6. Stravitz R T, Kramer D J. Management of acute liver failure[J]. Nat Rev Gastroenterol Hepatol, 2009, 6(9):542-553.
7. Bernal W, Jalan R, Quaglia A et al. Acute-on-chronic liver failure. The Lancet. 2015;386(10003):1576-1587.
8. Zhao-Ni, Lin,Yong-Qing, Zuo,Peng, Hu. Association of Proton Pump Inhibitor Therapy with Hepatic Encephalopathy in Hepatitis B Virus-related Acute-on-Chronic Liver Failure.[J].Hepatitis monthly,2014,14(4):e16258.

③ **Reviewer's comment:** "As authors report correctly, the presence of MHE was not assessed in all studies analyzed and this could understimate the risk of overall HE. This is in my opinion a great limitation of the study."

Response: It is really true as reviewer suggested that the lack of data regarding minimal HE may lead to an underestimation of the risk of overall HE. We have discussed this issue in our discussion section **(See revised manuscript Page 13, Line 358-364)**. In addition, we also have added this point to the discussion as part of the study's limitations.

See revised manuscript Page 16, Line 439-440

"The incidence of minimal HE was not assessed in most included studies, which might underestimate the risk of overall HE in PPI users."

Reviewer #2:

Special thanks to Reviewer#2 for these very useful comments. Below is our point-by-point response.

① **Reviewer's comment:** "The prevalence of H. pylori infection and the degree of gastric atrophy differ among various countries. H. pylori infection causes lower expression of proton pump and successful eradication increases its expression. The effect of PPI is different between H. pylori negative and positive subjects. These factors influence largely the level of acid secretion and the effect of proton pump inhibitors. Therefore, authors should focus on these factors to review an association of proton pump inhibitors with the risk of hepatic encephalopathy, even if direct association is not demonstrated."

Response:

We agree with the reviewer that data differences in different countries or studies may be affected by the effect of proton pump inhibitors. We also reviewed the relevant literatures.

According to the reviewer's suggestions, we have added some discussions on this point in our discussion section.

See revised manuscript Page 13, Line 347-351

"In addition, infection and eradication status of H. pylori and the degree of gastric atrophy may affect expression of proton pump. These factors largely influence gastric acid secretion and the effect of PPIs, thus causing differences in risk estimates in different locations. However, the impact of these factors cannot be analyzed because the data is not available."

② **Reviewer's comment:** "Proton pump inhibitors are mainly metabolized by liver metabolizing isozyme CYP2C19 including extensive metabolizer, poor metabolizer and intermediate type. Tsai CF et al. reported that rabeprazole is not associated with an increased risk of hepatic encephalopathy. Therefore, it is difficult to unify easily such an association among all proton pump inhibitors. Please describe possible association between this isozyme and effect of PPI."

Response:

Tsai CF et al. found that all individual PPIs, except rabeprazole, displayed an increased risk of HE occurrence. The small sample size of rabeprazole users may be one of the potential reasons. On the other hand, metabolic differences among different PPIs may be another possibility. This comment by the reviewer is very valuable, and we have added some discussions on this point in our discussion section.

See revised manuscript Page 15, Line 406-416

"Tsai CF et al. found that rabeprazole was not associated with an increased risk of HE. One reason may be that the sample size of rabeprazole users is too small, and another may be the metabolic difference among different types of PPIs. PPs are mainly metabolized in the liver by liver metabolizing isozyme CYP2C19. Based on different combinations of wild-type gene and mutated alleles, CYP2C19 genotypes can be classified as ultra rapid metabolizer, rapid metaboliser, intermediate metaboliser and poor metabolizer. Different genotypes can influence pharmacokinetics and acid-suppressive effect. In addition, individual PPI has its own unique metabolic pathway. These metabolic differences of PPIs may affect the occurrence of HE, and this deserves further research."

③ **Reviewer's comment:** "Introduction section. At present, several factors have been identified that lower the incidence of HE, such as infection, constipation, gastrointestinal bleeding, and the use of some nervous system drugs. Is this sentence correct?"

Response:

We are very sorry for our incorrect writing. We have re-written this sentence as "At present, several factors have been identified that induce the incidence of HE, such as infection, constipation, gastrointestinal bleeding, and the use of some nervous system drugs"**(See revised manuscript Page 5, Line 115-117)**

④ **Reviewer's comment:** "Introduction section. High-quality evidence from Europe and

China was published in 2018. Authors refer to a lot article in this review from various areas.Therefore, this sentence should be deleted."

Response:

We have deleted this sentence according to the Reviewer's comments**(See revised manuscript Page 5, Line 132-133)**

Reviewer#3:

We are deeply grateful to Reviewer#3 for taking the time to provide quite valuable comments and suggestions.

① **Reviewer's comment:** "Comment 1. Methods, P5, line 9: Please clearly describe when is the date of database inception."

Response:

We have made correction according to the Reviewer's comments.

See revised manuscript Page 6, Line 144-148

"Electronic databases including PubMed(from 1946 through January 8, 2019), EMBASE(from 1988 through January 8, 2019), Cochrane Central Register of Controlled Trials (CENTRAL) (from 1991through January 8, 2019), and Cochrane Database and Systematic Reviews (from 2005 through January 8, 2019) were searched by using subject headings and keywords."

② **Reviewer's comment:** "Comment 2. P6, Methods: What were the purposes of PPI use (prophylactic use or non-prophylactic use) in these included studies? How many studies included patients using PPIs for prophylaxis of some diseases?"

Response:

Six full-text studies reported indication for PPI treatment in different ways. The following table details the indication for PPI treatment. As can be seen from the following table, the indication for PPI treatment in each study included both prophylactic and non-prophylactic use. No studies only included patients using PPIs for prophylaxis of some diseases.

Author;Year	Indication for PPI treatment	HE group	Non-HE Group	P Value
Sturm et al 2018	Gastric ulcer	9.2%		-
	GERD	9.2%		-
	Variceal hemorrhage	21.8%		-
	NSAID comedication	0.7%		-
	Unknown	59.1%		-
Nardelli et al 2018	Recent gastrointestinal bleeding, recent endoscopic ligation of varices and severe reflux or peptic ulcer	41.6%		-

	disease			
	epigastric pain or abdominal discomfort	58.4 %		-
Zhu et al 2018	Upper gastrointestinal bleeding	48.0%	36.4%	0.180
	Peptic ulcer	2.9%	1.8%	1.000
	Prophylactic use of PPI for portal hypertension bleeding	6.9%	10.9%	0.380
	Prophylactic use of PPI for stress ulcer and bleeding	1.0%	0%	1.000
	Unclear indications	41.2%	50.9%	0.313
Tsai et al 2016	upper GI bleeding	73.3 %	57.3%	-
	peptic ulcer disease	60.9 %	59.6%	-
	gastroesophageal reflux disease/reflux esophagitis	19.8 %	23.8%	-
	others	8.7%	5.8%	-
Dam et al 2016	acid-related (ulceration, reflux, or esophagitis)	56%		-
	bleeding related (bleeding prophylaxis or after banding procedures)	10%		-
	cirrhosis condition (portal hypertension or ascites)	7%		-
	gastro-protection, heartburn,or nausea	27%		-
Lin et al 2014	GERD	2%	2.9%	1.000
	Peptic ulcer	4.1%	8.6%	0.555
	Prophylactic use of PPI for the portal hypertension bleeding	16.3%	25.7%	0.223
	Upper gastrointestinal bleeding	8.2%	5.7%	0.600
	No documented indication	69.4%	57.1%	0.175

③ **Reviewer's comment:** "Comment 3. P22, Results: Please show the Forest plot

evaluating the association between PPI and hepatic encephalopathy with stratification analyses based on prophylactic and non-prophylactic uses of PPIs."

Response:

As mentioned above, the indication for PPI treatment in each study included both prophylactic and non-prophylactic use. In addition, none of the six studies reported subgroup data on the risk of hepatic encephalopathy according to different indications. Therefore, we are unable to provide the forest plot evaluating the association between PPI and hepatic encephalopathy with stratification analyses based on prophylactic and non-prophylactic uses of PPIs.

We have added this point as a limitation of this meta-analysis in our discussion section.

See revised manuscript Page 16, Line 441-446

"Finally, due to insufficient data, we were unable to explore the association between the risk of HE and the type of PPI, indication for PPI treatment, time of PPI treatment, or method of PPI administration (oral/intravenous). We cannot predict whether the risk of HE changes after discontinuing the use of PPIs. These problems urgently need to be addressed in future studies."

④ **Reviewer's comment:** "Comment 4. P22, Results, P22, Figure 5. The rate ratios of in-hospital case control studies and current-definition cohort studies are quite different (3.51 vs 1.36). Please explain the difference in the Discussion section. The rate ratios concerning the association between PPI use and hepatic encephalopathy in the in-hospital case control studies might be over-estimated because these studies included patients using PPIs for upper gastrointestinal bleeding, and upper gastrointestinal bleeding was a risk factor of hepatic encephalopathy."

Response:

As the reviewer comment, the rate ratios of in-hospital case control studies and current-definition cohort studies are quite different. As we can see from Figure 5, the difference in each subgroup data may come mainly from the different study designs. Case-control designs can generate an exaggerated risk estimate because they are susceptible to various biases[9]. Furthermore, odds ratios (ORs) are usually calculated as the effect size of the case-control study. Due to its special algorithm, odds ratios (ORs) may overestimate the true effect of an exposure on the outcome of interest[10]. We have added some discussions on this point in our discussion section.

We agree with the reviewer that gastrointestinal bleeding is an important confounding factor, which may influence the association between PPI use and hepatic encephalopathy. However, the six studies included by our analysis have regarded gastrointestinal bleeding as an important confounding factor, and conducted univariate or multivariate analysis for this factor. Hence the risk of hepatic encephalopathy may not be affected by gastrointestinal bleeding.

See revised manuscript Page 13, Line 341-347

"It is worth noting that the pooled effect size of the case-control design was greater than that of the cohort design. Case-control designs can generate an exaggerated risk estimate because they are susceptible to various biases.[28] Furthermore, odds ratios (ORs) may overestimate the true effect of an exposure on the outcome of interest. We also found that the pooled risk estimates differed based on definition of PPI use, study location and type of advanced liver disease. These differences may be mainly due to different study designs."

Reference:

9. Schulz KF, Grimes DA. Case-control studies: research in reverse. The Lancet. 2002;359:431-434.

10. Schmidt C O , Kohlmann T . When to use the odds ratio or the relative risk?[J]. International Journal of Public Health, 2008, 53(3):165-167.

⑤ **Reviewer's comment:** "Comment 5. Discussion: Do the results of this meta-analysis change the clinical practice for PPI use in patients with advanced disease? What are the scenarios that PPIs are over-used in current practice?"

Response:

In patients with liver cirrhosis, inadequate indications for PPI therapy usually include, but are not limited to, the following: using PPIs for the treatment or prevention of variceal bleeding; long-term prophylactic treatment with PPIs after endoscopic variceal ligation; nonspecific symptoms such as abdominal pain and dyspepsia were also common inadequate indications; PPIs therapy is not discontinued after adequate treatment control and/or NSAID removal and/or H. pylori eradication[11,12]. Any of these treatment options does not constitute an adequate evidence-based indication and is not recommended by current guidelines.

Our meta-analysis included all previous studies on the association between PPI use and hepatic encephalopathy. However, the meta-analysis has some limitations, such as the small number of studies included, and most of the studies included are retrospective designs, etc. As a result, the quality of evidence is not high. The conclusion of this meta-analysis may not completely change clinical practice, but can be used as an evidence based on evidence-based medicine. More high-quality prospective studies are still needed to assess the association of HE with PPI treatment in cirrhotic patients.

Reference:

11. Kalaitzakis E , Bj?Rnsson E . Inadequate use of proton-pump inhibitors in patients with liver cirrhosis[J]. European Journal of Gastroenterology & Hepatology, 2008, 20(6):512-518.

12. Chavez-Tapia N C , Tellez-Avila F I , Garcia-Leiva J , et al. Use and overuse of proton pump inhibitors in cirrhotic patients[J]. Medical science monitor: international medical journal of experimental and clinical research, 2008, 14(9):CR468-72.

⑥ **Reviewer's comment:** "Comment 6. Discussion: Please include selection bias as a limitation in these included studies."

Response: We have added this point in our discussion section.

See revised manuscript Page 16, Line 440-441

“Some studies included special patients, which may lead to selection bias”

We also have made some changes in the manuscript according to the editor's requirements, and the modified part can be easily identified because we adopted the Microsoft Word review mode. We tried our best to revise our manuscript. However, we are very sorry that our figures(Figure 2-9) cannot be edited. These figures were exported directly from the Review Manager 5.3 software, and the function of this software does not support changes to image text and format. Besides, in the reference section, some DOIs and/or PMIDs cannot be found.

We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the correction will meet with approval. If some contents or formats cannot meet the requirements, we are very willing to revise our manuscript once again.

Thank you very much for your consideration and time.

Yours sincerely,

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