PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Changes in gastric mucosal microbiota in gastric carcinogenesis: a systematic review protocol
AUTHORS	Ji, Ruoyu; Zhao, Xinyu; Cao, Xinyuan; Zhang, Yizhen; Yang, Yingyun

VERSION 1 – REVIEW

REVIEWER	Yunsheng Yang and Wang Zikai Department of Gastroenterology and Hepatology, The First Medical Center, Chinese PLA General Hospital, Beijing 100853, China
REVIEW RETURNED	14-Nov-2020

GENERAL COMMENTS	It is important to define the characteristics of gastric microbiota in gastric carcinogenesis. H. pylori influences the composition of gastric microbiota, the autors should further define the gastric microbial characteristics of gastric cancer between H. pylori infection and non- H. pylori infection subjects. Moreover, the authors should make the detailed protocol to find out the differences among patients with gastric cancer, healthy subject with normal gastric mucosa, patients with superficial gastritis, atrophic gastritis and intestinal metaplasia. In addition, English language and wording need to be elaborated, and language editing is highly warranted.

REVIEWER	Xia Ding Beijing University of Chinese Medicine, Beijing, China.
REVIEW RETURNED	16-Nov-2020

GENERAL COMMENTS	This is an interesting and valuable study. Nevertheless, I have a number of concerns about this trial, and perhaps my comments may draw attention to what I consider deficiencies in the design and planned analysis. 1、Diagnostic criteria should be defined for patients with gastric cancer and non-cancer lesions (non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia). 2、As the author mentioned, the composition of gastric microbiota is dynamic and complex, as it can be impacted by several factors (such as gender, age and combined disease, etc) and differs geographically and ethnically. How to balance the influence of these factors on gastric microbiota should be clarified by the author. 3、The quality of the original research is critical to the results of the systematic review, and more details of the quality evaluation of the

	original research should be considered and reported. 4、The authors should pay attention to grammar and language mistakes among the whole manuscript.

VERSION 1 – AUTHOR RESPONSE

Response to the comments of Reviewer 1:

Dr. Yunsheng Yang

1. **The authors should make the detailed protocol to find out the differences among patients with gastric cancer, healthy subject with normal gastric mucosa, patients with superficial gastritis, atrophic gastritis and intestinal metaplasia.**

Response:

Thank you for the comment. The initial objective of this study is to identify the differences in the gastric microbiota profile between gastric cancer and non-cancer patients. However, as mentioned by the reviewer, the non-cancer group can be further subdivided into five histological stages (normal mucosa, non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia), and your great work in 2020 demonstrated that gastric microbiota changed progressively cross stages of gastric carcinogenesis. Hence, comparing the gastric microbiota between each of the non-cancer histological stages with cancer group respectively may help to understand when and how the gastric microbiota change along the normal to cancer cascade.

Therefore, the following modifications have been made.

1) Firstly, we have added the clear definition of each histological stage based on appropriate literatures in Methods and Analysis section, Study characteristics part:

For histological evaluation, the gastric cancer should be confirmed as gastric adenocarcinoma. Histological diagnoses of non-cancer histological types including normal gastric mucosa, non-atrophic gastritis, atrophic gastritis and intestinal metaplasia shall comply with updated Sydney System.[1] Accordingly, normal gastric mucosa is defined as normal epithelium and glandular compartments with only individual scattered chronic inflammatory cells. Non-atrophic gastritis is defined as increased infiltration of chronic inflammatory cells without loss of gastric glands proper. Atrophic gastritis is defined as loss of gastric glands proper. Intestinal metaplasia is defined as the presence of goblet cells, absorptive cells, and cells resembling colonocytes in the area of glands and mucosal epithelium. The diagnosis of intraepithelial neoplasia should be confirmed by revised Vienna classification system.[2]

2) Secondly, we have clarified that the extraction of patient characteristics and outcome data should be respectively performed in the cancer group and each histological type of non-cancer group in Methods and Analysis section, Data extraction and management part:

We will retrieve patient characteristics and outcome data in the cancer group and each histological type of non-cancer group, respectively.

3) Thirdly, we have detailed the comparison between each non-cancer histological type with the cancer group in Methods and Analysis section, Data synthesis and statistical analysis part:

Additionally, we will compare the differences in alpha diversity indexes and relative abundance of bacterial phyla or genera between each non-cancer histological type (normal mucosa, non-atrophic gastritis, atrophic gastritis, intestinal metaplasia, intraepithelial neoplasia) and the cancer group, respectively.

2. *H. pylori* influences the composition of gastric microbiota, the authors should further define the gastric microbial characteristics of gastric cancer between *H. pylori infection* and *non- H. pylori* infection subjects.

Response:

Thank you for the comment, and we are inspired by your comment. We agree with the importance to balance the influence of *H. pylori* infection on gastric microbiota and to explore the interstudy heterogeneity brought by *H. pylori* infection. Therefore, the following modifications have been made.

1) Firstly, we have added the definition of *H. pylori* infection status in Methods and Analysis section, Study characteristics part:

The H. pylori infection status is determined on the basis of ^{13}C urea breath test or histological assessment.

2) Secondly, we have we have supplemented the extraction of *H. pylori* infection status in Methods and Analysis section, Data collection and analysis part:

Patient characteristics: demographics (age, sex, country or region, race/ethnicity, comorbidities), lesion location, clinical and histological diagnosis and H. pylori infection status.

3) Thirdly, we have added a subgroup analysis based on *H. pylori* infection status in Methods and Analysis section, Data synthesis and statistical analysis part:

We will conduct the following subgroup analyses to explore potential sources of heterogeneity: age, sex, race/ethnicity, comorbidities, country or region, H. pylori infection status, source of samples and sample size. Meta-regression will be performed to identify sources of heterogeneity across studies.

3. In addition, English language and wording need to be elaborated, and language editing is highly warranted.

Response:

Thank you for your criticism and suggestion. As requested, we have proofread the manuscript carefully and fixed the grammar mistakes and typos. In addition, we have improved the language with the assistance from a native English speaker.

Response to the comments of Reviewer 2:

Dr. Xia Ding

1. **Diagnostic criteria should be defined for patients with gastric cancer and non-cancer lesions (non-atrophic gastritis, atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia).**

Response:

Thank you for the comment. We do agree with that the detailed definition of gastric cancer and non-cancer lesions, especially different histological types of non-cancer group, is necessary for this systematic review. Therefore, we have added the definition of each histological diagnosis based on the appropriate literature in Methods and Analysis section, Study characteristics part:

For histological evaluation, the gastric cancer should be confirmed as gastric adenocarcinoma. Histological diagnoses of non-cancer histological types including normal gastric mucosa, non-atrophic gastritis, atrophic gastritis and intestinal metaplasia shall comply with updated Sydney System.[1] Accordingly, normal gastric mucosa is defined as normal epithelium and glandular compartments with only individual scattered chronic inflammatory cells. Non-atrophic gastritis is defined as increased infiltration of chronic inflammatory cells without loss of gastric glands proper. Atrophic gastritis is defined as loss of gastric glands proper. Intestinal metaplasia is defined as the presence of goblet cells, absorptive cells, and cells resembling colonocytes in the area of glands and mucosal epithelium. The diagnosis of intraepithelial neoplasia should be confirmed by revised Vienna classification system.[2]

2. As the author mentioned, the composition of gastric microbiota is dynamic and complex, as it can be impacted by several factors (such as gender, age and combined disease, etc) and differs geographically and ethnically. How to balance the influence of these factors on gastric microbiota should be clarified by the author.

Response:

Thank you for the comment, and we were inspired by your comment. The gastric microbiota can be impacted by a series of factors and these factors may be potential sources of heterogeneity. To balance the influence of these factors on gastric microbiota and to identify the source of heterogeneity, we thus consider it necessary to perform subgroup analyses based on several factors

(age, sex, race/ethnicity, combined disease, country or region, sample size) that are routinely analyzed and some other factors (*H. pylori* infection status, source of samples) that may exert an impact on the composition of gastric microbiota as suggested by literatures. Moreover, meta-regression is planned to further determine the source of heterogeneity. Therefore, the following modifications have been made:

1) Firstly, we have supplemented and modified the extraction of relevant data that are analyzed in subgroup analyses in Methods and Analysis section, Data extraction and management part:

Information of the study: publication (authors, year, journal title, format), study design (patient inclusion and exclusion criteria, source of samples, grouping and the sample size of each, sequencing technology), bias control.

Patient characteristics: demographics (age, sex, country or region, race/ethnicity, comorbidities), lesion location, clinical and histological diagnosis and H. pylori infection status.

2) Secondly, subgroup analyses have been added in Methods and Analysis section, Data collection and analysis, Data synthesis and statistical analysis part:

We will conduct the following subgroup analyses to explore potential sources of heterogeneity: age, sex, race/ethnicity, comorbidities, country or region, H. pylori infection status, source of samples and sample size. Meta-regression will be performed to identify sources of heterogeneity across studies.

3. The quality of the original research is critical to the results of the systematic review, and more details of the quality evaluation of the original research should be considered and reported.

Response:

Thank you so much for the suggestion. Inspired by your comment, we read other high-quality systematic reviews and protocols and we found it necessary to elaborately modify the NOS to make it a more suitable tool for our research. We then read the full text of several original articles that may be included in the further meta-analysis, and other systematic reviews evaluating gut microbiota. We have supplemented and adapted the detailed criteria in NOS with the intention of best evaluating our phenomenon of interest. Therefore, the following modifications have been made in Methods and Analysis section, Risk of bias assessment part:

We will assess the risk of bias using a modified Newcastle-Ottawa Scale (NOS) (supplementary appendix 2). NOS is a scoring system designed to evaluate the risk of bias in non-randomized studies, and we have incorporated adaptations based on the original version[3] with the intention of best evaluating our phenomenon of interest. The modified NOS additionally considers the following aspects: a) subdivision of non-cancer lesions into normal gastric mucosa, non-atrophic gastritis,

atrophic gastritis, intestinal metaplasia and intraepithelial neoplasia according to histological evaluation, b) clear exclusion criteria to prevent the impact of surgery or drugs on gastric microbiota, c) sample size, d) adjusting for H.pylori infection status and other demographic characteristics in analyses, e) description of detailed procedures and quality control of experiments. The assessment will be evaluated from three domains: selection, comparability and exposure (or outcome), and each study will be awarded with a maximum of 11 scores.

4. **The authors should pay attention to grammar and language mistakes among the whole manuscript.**

Response:

Thank you for your criticism. As suggested, we have gone through the manuscript carefully and fixed the grammar mistakes and typos. In addition, we have improved the language with the assistance from a native English speaker.

VERSION 2 – REVIEW

REVIEWER	dr. yang yunsheng Department of Gastroenterology and Hepatology, The First Medical Centre, Chinese PLA General Hospital, Beijing, China
REVIEW RETURNED	12-Feb-2021

GENERAL COMMENTS	The authors have revised this manuscript according to the reviewers' suggestion, and this manuscript could be considered for acceptance after editorial review.