To the Science Editor,

Editorial Office

BAISHIDENG PUBLISHING GROUP INC

Dear Professor Jia-Ping Yan,

We would like to kindly thank you and the reviewers for the time spent on reviewing our manuscript and for comments helping us improving the article. Please find attached a revised version of our manuscript entitled “Helicobacter pylori and cytokine gene variants as predictors of premalignant gastric lesions” by Anca Negovan, Mihaela Iancu, Emőke Fülöp, Claudia Bănescu, manuscript number: 47633.

Following the reviewer’s observations, we made some modifications to the initial version of our manuscript (in red colour), which we described point-by-point, according to recommendations, as follows.

Reviewer’s code: 00717554

“I would like to mention the following comments: “

Comment 1

“1-The first sentences of abstract (fifth) and introduction (third) are confusing, although one is death and one is diagnosed.”
Response 1:

We introduce the same data regarding gastric cancer mortality in both Abstract and Introduction chapters.

Comment 2

“2- Epidemiology: incidence and mortality rates are missing.”

Response 2:

We added a phrase in red from the same source regarding the incidence of gastric cancer worldwide.

Comment 3

"3- It might be better to explain the different parts of text to at least 3 parts."

Response 3

We do not understand the recommendation.

Comment 4

"4- Table 1: Statistical methods or models? It is just methods and the name of tests". Good Luck

Response 4

We synthetize the methods of the studies. We change the incorrect use of the term.

Reviewer's code: 00058340

"The authors reviewed the role of cytokines and their gene variants in regard to non-self-limiting H. pylori gastritis and its evolution to gastric atrophy and intestinal metaplasia; the literature now includes various and non-conclusive results on this topic. While the influence of the majority of cytokine single nucleotide polymorphisms has been investigated for gastric cancer it was not examined for preneoplastic gastric lesions. Amongst the investigated gene variants only IL10T-819C, IL-8-251, IL-18RAP917997, IL-22 rs1179251, IL1-B-511, IL1-B-3954, IL4R-398 and IL1RN were identified as predictors for premalignant gastric lesions risk. The review is good and comprehensive but in order to provide a more in depth insight and a full picture the authors should add important information as follow: "

Comments 1

"a) Gastric intestinal metaplasia (IM) and gastric cancer are associated with Helicobacter pylori, but the bacterium often is undetectable in these lesions. However, H. pylori and its genes were detected inside metaplastic, dysplastic, and neoplastic epithelial cells, and cagA and babA2 expression was colocalized. The preneoplastic "acidic" MUC2 mucin was detected only in the presence of H. pylori, and MUC2 expression was higher in patients with IM, dysplasia, and cancer. These findings are compatible with the hypothesis that all stages of gastric carcinogenesis are fostered by persistent intracellular expression of H. pylori virulence genes, especially cagA inside MUC2-producing precancerous gastric cells and pleomorphic cancer cells. Semino-Mora C, et al. Intracellular and interstitial expression of Helicobacter pylori virulence genes in gastric precancerous intestinal metaplasia and adenocarcinoma. J Infect Dis. 2003 Apr 15;187(8):1165-77. PMID: 12695995"

Response 1

We added a paragraph in red containing the information and we added the mentioned reference. We agree that the data represent the possible link between persistence and virulence of *Helicobacter pylori* infection and the risk for gastric cancer and offer a more comprehensive picture of the issue.

Comment 2

"b) Important studies, e.g. one cited below demonstrated that chronic infection of C57BL/6 mice with Helicobacter, a known carcinogen, induces repopulation of the stomach with BMDCs. Subsequently, these cells progress through metaplasia and dysplasia to intraepithelial cancer. These findings suggest that epithelial cancers can originate from marrow-derived sources and thus have broad implications for the multistep model of cancer progression. Several cytokines are critical for recruitment of bone marrow-derived progenitor cells. Houghton et al. Gastric cancer originating from bone marrow-derived cells. Science. 2004 Nov 26;306(5701):1568-71."

Response 2

We introduced a sentence in red stated this possible link between infection and progression toward metaplasia proved in mice models and we added the recommended reference.

Comment 3

"c) accumulating evidence indicates that stem cells are the major cellular origin of most cancers, including gastric cancer. Hayakawa Y et al. The Origins of Gastric Cancer From Gastric Stem Cells: Lessons From Mouse Models. Cell Mol Gastroenterol Hepatol. 2017 May; 3(3): 331–338. PMID: 28462375"

Response 3.

We added a sentence and a reference in red regarding the role of aberrant stem cell in gastric cancer.

Reviewer's code: 02954663

"The manuscript is a well-written narrative review on the role of cytokines - albeit a little bit exhaustive- and gastric premalignant conditions. The title and abstract are adequate, the keyword are well chosen and a main body of the manuscript is well systematized. However, the information is overwhelming and ther manuascript does not present the practical importance of cyítokine measurement. I strongly recommend to the authors to present and comment the following:

Comment 1

"a) is there any correelation between OLGA/OLGIM staging and level of any cytokine?"

Response 1

The complex pathophysiologic roles of many cytokines unfortunately remain undefined despite the progression made in understanding some of their role in gastric carcinogenesis. There are not published studies correlating the level of cytokine and the presence of histological gastric changes based on OLGA/OLGIM systems. Except from the studies regarding the polymorphisms of cytokine presented in our review, there are published studies correlating gastritis, intestinal metaplasia, gastric atrophy and gastric cancer with the cytokine level, but not using mentioned grading and staging systems. We added a sentence emphasizing this aspect and the most recent reference synthetizing the biological studies in humans in relationship with premalignant gastric lesions.

Comment 2

b) is there anyí correelation between serologic bioosy (pepsinogen I/II nratio + H pylori + gastrin 127) and level of certain cytokines?

Response 2

The current guideline stated that low pepsinogen I/II ratio identify patients with advanced stages of atrophic gastritis and only endoscopy is recommended for these patients, particularly if *H. pylori* serology is negative. There are several published studies correlating the serum level of pepsinogen I or pepsinogen I/II ratio with serological levels of various cytokine (IL-IB, IL-17, etc.), but not with all "serologic biopsy" components (pepsinogen I/II ratio + H pylori + gastrin 127). The majority of published studies in this respect are performed in patients with gastric atrophy on histology for gastric cancer risk assessment. Our paper focus only on genetic polymorphisms of cytokine that were proved to play a role in host immune response involved in gastric carcinogenesis, not questioning the correlation of their expression with premalignant conditions (gastric atrophy). We agree that the practical effect of these researches is the possible serologic diagnosis of patients on cancer risk combining several biologic markers.

Comment 3

c) what is the place of cytokine measurements - excepting research - in the routine diagnosis and management of precancerous conditoons; are these methods included in the most recent quidelines (ex. Nunes PP et al., Endoscopy, doi: 10.-1055/a-0859-1883)

Response 3

Cytokines have pleiotropic effects on various cell types and regulate several molecular processes during chronic inflammation and seems to be involved in the progression from gastric inflammation toward gastric cancers. For the moment we agree that there are not robust evidence to support the routine examination of cytokine level for surveillance the patients with precancerous conditions. The current of guideline does not mention their practical role, as it has not been proved in published researches.

Yours sincerely,

Anca Negovan