

Perugia, December 03, 2022

Editor-in-Chief of World Journal of Gastroenterology.

Dear Prof. Ma,

Please find enclosed the revised manuscript entitled **“THE CYTOTOXIC SYNERGISM OF *CLOSTRIDIODES DIFFICILE* TOXIN B WITH PROINFLAMMATORY CYTOKINES IN SUBJECTS WITH INFLAMMATORY BOWEL DISEASES”** by Gabrio Bassotti, Alessandro Fruganti, Fabrizio Stracci, Pierfrancesco Marconi and Katia Fettucciari. We have revised the Manuscript NO.: 80059, Review, according to the Peer-Review Report, Editorial Office’s comments and the Criteria for Manuscript Revision.

We sincerely appreciate the valuable comments and suggestions from the Editorial office’s and Reviewers. The thorough review helped strongly in reshaping the manuscript. The suggestions and comments have been closely followed, and revisions have been made accordingly. The followings are the questions extracted from the reviewers' comments and our point-by-point responses.

Reviewer #1:

Language Quality: Grade B (Minor language polishing)

AUTHOR ANSWER:

Thank you for appreciation of our article. We performed further language polishing to ensure that grammatical, syntactical, formatting and other related errors were resolved, so that the revised manuscript now meets publication requirements (Grade A).

Reviewer #2:

Language Quality: Grade B (Minor language polishing)

AUTHOR ANSWER:

Thank you for appreciation of our article. We performed further language polishing to ensure that grammatical, syntactical, formatting, and other related errors were resolved, so that the revised manuscript now meets publication requirements (Grade A).

Reviewer #3:

1. The content of the article is not very relevant to the topic, only a few paragraphs mention the synergistic effect of *Clostridium difficile* toxin B and proinflammatory cytokines.

AUTHOR ANSWER:

We agree with the Reviewer when he says that a few paragraphs have been dedicated to the synergistic cytotoxic effect of TcdB+CKs. This is due to the following considerations:

- a. To adequately explain the phenomenon of cytotoxic synergism between TcdB+CKs, we thought of importance to describe the most recent features of the mechanism of action of TcdB, starting from the interaction with its membrane receptors, and then with the activated signalling pathways of cell death.
- b. We have paid special emphasis to our innovative hypothesis with important clinical implications that the CKs can induce a greater expression in the colon of subjects with IBD of the receptors for TcdB which mainly activate necrotic cell death.
- c. Despite the clinical importance of *C. difficile* infections in individuals with IBD, there are only a few contributions in the literature that attempt to explain the higher incidence and causes of more severe consequences of *C. difficile* infection in these individuals, aspect that we have commented in the paragraph **“THE CONDITIONS THAT FAVOUR THE ENDEMIC SPREAD OF C. DIFFICILE IN PEOPLE WITH IBD.”**

2. Some sentences are too long to read easily, such as "It is clear that if CDI occurs in a subject with IBD the clinical picture can progress to a more acute form for the expression of high levels of receptors for TcdB that induce cell death by necrosis and higher levels of cytokines that enhance the development of receptors for TcdB, inducing mainly necrosis and enhancing the cell death by apoptosis/necrosis."

AUTHOR ANSWER:

We agree with the Reviewer that several sentences were too long; therefore we have intervened on several sentences to break up the text, for a easier reading.

3. The part of "Tcds uptake, internalization and intracellular molecular effects" is too long, which should be divided.

AUTHOR ANSWER:

We agree with the Reviewer that the part of manuscript "Tcds uptake, internalization and intracellular molecular effects" is too long; therefore we have divided it into the following brief sub-chapters which thus allow a greater focus on the concepts.

- i. Tcds uptake and internalization.
- ii. Tcds intracellular effects.

- iii. Tcds effect in vivo.
- iv. Tcds and cell death.

4.The content of current clinical status, treatment methods and strategies is less, it is suggested to be supplemented.

AUTHOR ANSWER:

We have now supplemented the content of current clinical status, treatment methods and strategies in "*Perspectives for future approaches to CDI treatment*".

EDITORIAL OFFICE'S COMMENTS

(2) Company editor-in-chief:

- According to the request we have added **Figure 1** to the manuscript.
- According to the request we send Audio for Core Tips.
- We have added "latest highlight articles" to improve our article: **New References: 114-118.**

We are grateful to the Editor for his encouraging suggestions to revise our study which has allowed us to improve the manuscript.

We hope that the manuscript is now suitable for publication in World Journal of Gastroenterology.

Yours sincerely,

Professor Gabrio Bassotti