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July 8, 2021

The Editor in Chief,

World Journal of Gastroenterology Pathophysiology,

Dear Dr. Somchai Amornyotin,

Resubmission: Revised manuscript 66391_ID04092906

Thank you for the opportunity to submit revisions for the article titled my article ""**Chemokine Receptor 8 Expression may be Linked to Disease Severity and Elevated Interleukin 6 Secretion in Acute Pancreatitis**" for publication in the World Journal of Gastroenterology Pathophysiology.

We have addressed the comments made by the one reviewer in the "Answering Reviewers" document and have incorporated some of these in the manuscript. We found the reviewer's comments useful in improving the manuscript. All other editorial requests have been addressed accordingly.

We look forward to working with you to move this paper closer to print

Yours sincerely,

Dr Pascaline N Fonteh, FRU
BSc., MSc, Ph.D. Biochemistry,
Senior Research Scientist and Research Coordinator

Responses to Reviewer comments

Reviewer 1

Specific Comments to Authors: In this study, the authors aimed to identify the role of the CCR8 and its possible association to IL-6 as early markers to assist with AP stratification. The study showed that CCR8 was shown to increase steadily with disease severity from MAP (1.33), MSAP (38.28) to SAP (1172.45) and suggests a possible linkage between increasing CCR8 expression and disease severity.

Request
However, the sample size of this study is very small, which makes a lot of statistical analysis impossible. Since acute pancreatitis is a common disease, the sample size of each group needs to be expanded.

Response
We would like to thank the reviewer for this comment. We realize that the sample size is small especially for severe group of patients in the RT2 profiler study, which we acknowledge in the paper (page 21 lines 578-589). However, for the mild and moderately severe patients, the samples size is reasonable and comparable to other published work within our demographics in South Africa. Thomson et al (2019) was able to recruit 36 patients over a one year period, whilst Anderson and Thomson (2017) recorded 627 patient with acute pancreatitis over a 9 year period (from May 2001 to November 2010) resulting in an annual recruitment of 63 patients and includes patients of all ethnicities as well as at all time points at presentation. This is unlike the current study, which focused on acute pancreatitis patients of black African descent who had to be within the first seven days post onset of pain.

Furthermore, other clinical data has previously published with similar numbers of patients as our study. Please see some examples below:

1. 12 patients; 19 controls – Histological examination of pancreas tissue of slowly progressive insulin-dependent diabetes mellitus (DOI: 10.1097/MPA.0000000000001144)
2. 38 patients - Factors that diminish a successful islet cell harvest during total pancreatectomy with islet autotransplantation (DOI: 10.1097/MPA.0000000000001116)
3. 17 patients - Contrast-enhanced ultrasonography after pancreas transplant (DOI: 10.1097/MPA.0000000000001051)
4. 30 patients - prospectively evaluated recurrence in PC cases diagnosed at an early stage (DOI: 10.1097/MPA.0000000000001021)
5. 45 patients - KRAS mutations detected in pancreatic juice samples (DOI: 10.1097/MPA.0000000000000956)

6. 28 patients – retrospective analysis (DOI: 10.1097/MPA.0000000000000919)

7. 39 patients – prospective questionnaire (DOI: 10.1097/MPA.0000000000000917)
8. 22 patients – health questionnaire/survey (DOI: 10.1097/MPA.0000000000000890)
9. 30 patients – comparison of magnetic resonance pancreatography (MRP) image quality (DOI: 10.1097/MPA.0000000000000853)
10. 26 patients – peripheral blood samples for circulating epithelial cells (DOI: 10.1097/MPA.0000000000000869)
11. 29 surgical specimens – CD63 and CD9 staining of pathological samples (DOI: 10.1097/MPA.0000000000000847)

In addition, to make preliminary conclusions to the genes that were analysed. We have also emphasized the fact that the work is preliminary. Again, the cytometric bead array data and the milliplex data has many more samples than the RT2 profiler and return results for IL-6 which are similar to literature findings and which we allude to. For this reason, we believe that the sample size is reasonable to make these preliminary conclusions given that our findings from the IL-6 work and lymphocytes frequency levels is supported by literature. Sampling for this project is ongoing and we plan to expand, as COVID-19 restrictions get easier by collecting more samples for an expanded study with bigger numbers.

A comment on this has been included in the manuscript page 21 lines 578-589

Request

Although the concentration of IL-6 and expression of CCR8 were analyzed at Day 1,3,5,7 post epigastric pain, their expression in the later stage of the disease might be mainly affected by treatment.

Response

The general treatment guideline for AP in the hospital unit is based on supportive care and none of this is known to affect immune responses. All patients are treated according to the same protocol. In mild AP, only analgesia and fluids are prescribed and nutrition is maintained with a combination of enteral and/or parenteral feeding. In the Moderate and severe group organ support is implemented depending on the organ dysfunction.

Antibiotics nor steroids are used routinely in the first phase of the inflammatory response in any of the patients and as such, we do not think that the treatment will influence the expression of IL-6 or CCR8 up to and including day 7.

A comment on this has been made in the manuscript on pages 21/22 lines 590 to 598

Request

4 LANGUAGE QUALITY

Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar,

sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

Response
A native-English speaker edited the manuscript for grammar. A certificate has been included with this submission to support this.

Request

5 ABBREVIATIONS
In general, do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly. Now we list the abbreviations rules as follows.

Request

(1) Title: Please spell out any abbreviation in the title. Abbreviations are not permitted.

Response

CCR8 and IL-6 have been spelt out in the title. The title now reads *Chemokine Receptor 8* Expression may be linked to Disease Severity and Elevated Interleukin 6 Secretion in Acute Pancreatitis

Request
(2) Running title: Please shorten the running title to no more than 6 words. Abbreviations are permitted.

Response
The running title has been shortened from: *CCR8* expression and IL-6 secretion for improved acute pancreatitis stratification and now reads "*CCR8* and IL-6 in AP severity."

See page 1 line 9.

Request

(3) Abstract: Abbreviations must be defined upon first appearance in the Abstract. Examples:
Example 1: Hepatocellular carcinoma (HCC). Example 2: Helicobacter pylori (H. pylori).

Response
All abbreviations have been defined upon first appearance in the abstract. Pages 3-4.

Request

(4) Key words: Abbreviations must be defined upon first appearance in the Key words.

Response
IL-6 and CCR8 have been defined in the keywords and they now read:

Acute pancreatitis; Severity; Stratification, Interleukin-6; Chemokine Receptor 8; Lymphocytes; Monocytes. See pages 4.

Request

(5) Core tip: Abbreviations must be defined upon first appearance in the Core tip. Examples:
Example 1: Hepatocellular carcinoma (HCC). Example 2: Helicobacter pylori (H. pylori)

Response
All abbreviations such as
CCR8, Th2, lymphoid cells group 2 and 3 (ILC2 and 3), MSAP, MAP, IL-6, lymphoid cells group 2 (ILC2) have been defined upon first use. Pages 4-5, lines 100-110

Request

(6) Main Text: Abbreviations must be defined upon first appearance in the Main Text. Examples:
Example 1: Hepatocellular carcinoma (HCC). Example 2: Helicobacter pylori (H. pylori)

Response
This has been verified and all abbreviations are defined upon first use.

Request

(7) Article Highlights: Abbreviations must be defined upon first appearance in the Article
Highlights. Examples: Example 1: Hepatocellular carcinoma (HCC).
Example 2: Helicobacter pylori (H. pylori)

Response
Abbreviations have been defined upon first appearance in the article highlights (pages 22 to 24 line 613-661)

(8) Figures: Please verify the abbreviations used in figures and define them (separated by semicolons) at the end of the figure legend or table; for example, BMI: Body mass index; CT: Computed tomography.

Response
Abbreviations have been defined and placed at the end of each Figure.

(9) Tables: Please verify the abbreviations used in tables and define them (separated by semicolons) at the end of the figure legend or table; for example, BMI: Body mass index; CT: Computed tomography.

Response
Abbreviations have been defined and placed at the end of the table

Request
6 EDITORIAL OFFICE'S COMMENTS
Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:
(1) Science editor: 1 Scientific quality: The manuscript describes the role of the CCR8 and its possible association to IL-6 as early markers to assist with AP stratification. The study revealed that CCR8 was shown to increase steadily with disease severity from MAP (1.33), MSAP (38.28) to SAP (1172.45) and suggests a possible linkage between increasing CCR8 expression and disease severity. The topic is within the scope of the WJG. (1) Classification: Grade D;
(2) Summary of the Peer-Review Report:
Request
Although the concentration of IL-6 and expression of CCR8 were analyzed at Day 1,3,5,7 post epigastric pain, their expression in the later stage of the disease might be mainly affected by treatment. The authors should explain these points.

Response
A response for this question has been provided above and reads

The general treatment guideline for AP in the hospital unit is based on supportive care and none of this is known to affect immune responses. All patients are treated according to the same protocol. In mild AP only analgesia and fluids are prescribed and nutrition is maintained with a combination of enteral and/or parenteral feeding. In the Moderate and severe group organ support is implemented depending on the organ dysfunction.

Antibiotics nor steroids are used routinely in the first phase of the inflammatory response in any of the patients and as such we do not think that the treatment will influence the expression of IL-6 or CCR8 up to and including day 7.

A comment on this has been made in the manuscript on page 21 lines 578-589

(3) Format: There are three tables and seven figures.

Response

No changes have been made to the number of tables and Figures.

Request
(4) References: A total of 52 references are cited, including 12 references published in the last three years;

Response
Not applicable

Request
(5) Self-cited references: There is no self-cited reference.

Response
The reviewer might have missed it but there are four self-citations in the reference list. The names are bolded. Fonteh is now Fru

1 **Fonteh P**, **Smith M**, Brand M. Adaptive Immune Cell Dysregulation and Role in Acute Pancreatitis Disease Progression and Treatment. Arch Immunol Ther Exp (Warsz). 2018; **66**:199–209.

2 Thomson J-E, Brand M, **Fonteh P**. The immune imbalance in the second hit of pancreatitis is independent of IL-17A. *Pancreatology* 2018; **18:246**–52. [PMID: 29422392 DOI: 10.1016/j.pan.2018.01.007]

3 Kay PS, **Smith M,** Brand M. The Initiating Immune Response of Acute Pancreatitis May be Mediated by the T-Helper 17 Pathway. *Pancreas* 2017; **5** [PMID: not available DOI: not available]

4 Thomson J-E, **Nweke EE,** Brand M, Nel M, Candy GP, **Fonteh PN**. Transient Expression of Interleukin-21 in the Second Hit of Acute Pancreatitis May Potentiate Immune Paresis in Severe Acute Pancreatitis. *Pancreas* 2019; **48**:107–12. [PMID: 30451792 DOI: 10.1097/MPA.0000000000001207]

(6) References recommendations: No particular recommendations.
2 Language evaluation: Classification:
Grade B A language editing certificate issued by Dr. Pascaline N Fonteh, FRU BSc., MSc, Ph.D. Biochemistry was provided. The authors speak English as a first language.

Response
Not applicable

3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, Clinical Trial Registration Statement, the signed Conflict-of-Interest Disclosure Form and

Request
Copyright License Agreement, and the Institutional Review Board Approval Form were provided. 4 Supplementary comments: Funding; Non.

Response
There is funding for this Project from the National Research Foundation of South Africa and other university of the Witwatersrand entities. These were stipulated in the manuscript under the sub-heading "Supported by" and the grant letters have been submitted as proof.

The topic has not previously been published in the WJG.

Response
Not applicable

Request

5 Issues raised: 1. The sample size of this study is very small. 2. In spite of the concentration of IL-6 and expression of CCR8 were analyzed at Day 1,3,5,7 post epigastric pain, their expression in the later stage of the disease might be largely affected by treatment.

Response
This concern has been addressed above

Request
6 Re-Review: Not required. 7 Recommendation: Conditional acceptance

Response
Noted.

(2) Company editor-in-chief: I recommend the manuscript to be published in the World Journal of Gastrointestinal Pathophysiology.

Response
Noted.

7 STEPS FOR SUBMITTING THE REVISED MANUSCRIPT

Step 1: Author Information
Please click and download the Format for authorship, institution, and corresponding author guidelines, and further check if the authors names and institutions meet the requirements of the journal.

Response
This has been done

Step 2: Manuscript Information
Please check if the manuscript information is correct.

Response
This is done

Step 3: Abstract, Main Text, and Acknowledgements
(1) Guidelines for revising the content: Please download the guidelines for Original articles;
Review articles; and Case report articles for your specific manuscript type (Clinical and Translational Research) at: https://www.wjgnet.com/bpg/GerInfo/291. Please further revise your manuscript according to the guidelines for revising the content.

Response
This has been done

(2) Format for Manuscript Revision: Please update the format of your manuscript according to the guidelines and requirements for manuscript revision and the format for manuscript revision. please visit https://www.wjgnet.com/bpg/GerInfo/291 for the article type-specific guidelines and formatting examples.

Response
This is done

(3) Requirements for Article Highlights: If your manuscript is an original study (basic study or clinical study), meta-analysis, or systemic review, the "Article Highlights" section should be provided. Detailed writing requirements for the "Article Highlights" can be found in the Guidelines and Requirements for Manuscript Revision.

Response
This is done

Step 4: References
Please revise the references according to the Format for references guidelines, and be sure to edit the reference using the reference auto-analyser.

Response
This is done

Step 5: Footnotes and Figure Legends
(1) Requirements for Figures: Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file, and submit as "**66391- Figures.ppt**" on the system. The figures should be uploaded to the file destination of "Image File".

Response
This is done

(2) Requirements for Tables: Please provide decomposable Tables (in which all components are movable and editable), organize them into a single Word file, and submit as "**66391-Tables.docx**" on the system. The tables should be uploaded to the file destination of "Table File".

Response
This is done

Step 6: Automatically Generate Full Text Files
Authors cannot replace and upload the "Manuscript File" separately. Since we only accept a manuscript file that is automatically generated, please download the "Full Text File" or click "Preview" to ensure all the contents of the manuscript automatically generated by the system are correct and meet the requirements of the journal. If you find that there is content that needs to be modified in the Full Text File, please return to the corresponding step(s), modify and update the content, and save. At this point, you then have to click the "Save & Continue" button in Step 5 and the F6Publishing system will automatically regenerate the Full Text File, and it will be automatically stored.

Response
This is done

Step 7: Upload the Revision Files

For all required accompanying documents (listed below), you can begin the uploading process via the F6Publishing system. Then, please download all the uploaded documents to ensure all of them are correct.

Response
The following files have been provided accordingly.
(1) 66391-Answering Reviewers
(2) 66391-Audio Core Tip
(3) 66391-Biostatistics Review Certificate
(4) 66391-Clinical Trial Registration Statement
(5) 66391-Conflict-of-Interest Disclosure Form
(6) 66391-Copyright License Agreement
(7) 66391-Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s)
(8) 66391-Signed Informed Consent Form(s) or Document(s)
(9) 66391-Institutional Review Board Approval Form or Document
(10) 66391-Non-Native Speakers of English Editing Certificate
(11) 66391-Video
(12) 66391-Image File
(13) 66391-Table File
(14) 66391-Supplementary Material
If your manuscript has supportive foundations, the approved grant application form(s) or funding agency copy of any approval document(s) must be provided. Otherwise, we will delete the supportive foundations.
If your manuscript has no "Video" or "Supplementary Material", you do not need to submit those two types of documents.

8 COPYRIGHT LICENSE AGREEMENT
Please click and download the Copyright License Agreement Form. Subsequently, a PDF (scanned) version of the Copyright License Agreement Form that has been signed by all authors should be uploaded to the file destination of 'Copyright License Agreement'.

Response
This is done

9 CONFLICT-OF-INTEREST DISCLOSURE FORM
Please click and download the fillable ICMJE Form for Disclosure of Potential Conflicts of Interest (PDF), and fill it in. The Corresponding Author is responsible for filling out this form. Once filled out completely, the Conflict-of-Interest Disclosure Form should be uploaded to the file destination of 'Conflict-of-Interest Disclosure Form

Response

A sample conflict of interest form has been included as recommended.