August 16, 2014

Dear Editor,

World Journal of Gastroenterology

Please find enclosed the edited manuscript in Word format (file name: MCP1PlasmaLeve-ESPN Manuscript NO- 11021 Revised Version).

Title: Plasma Levels of Monocyte Chemotactic Protein-1 (MCP-1) Are Elevated in Colorectal Cancer Patients Preoperatively; After Resection Levels Are Further Increased and Remain Elevated For Over 1 Month

Author: Shantha Kumara HMC, Elizabeth A Myers, Sonali AC Herath , Joon Ho Jang, Linda Njoh , Xiaohong Yan , Daniel Kirchoff , Vesna Cekic, Martin Luchtefeld, Richard L Whelan

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 11021

The manuscript has been improved according to the suggestions of reviewers:

Manuscript NO: 11021

WJSO Reviewers Comments and replies to comments;

Reviewer 1:

The problem discussed in the paper sheds a light on plausible reason for the development of metastases after the resection of colorectal cancer. The paper is written by colorectal surgeons, professor Whelan and colleagues and are reporting their observations on plasma levels of MCP-1 before and after resection of CRC surgery for 1 month postoperatively and compared to BEN. Herewith they report that the elevation of MCP-1 observed postoperatively may promote angiogenesis, cancer recurrence and metastasis. Further, the authors believe that the cancer is not responsible for the postoperative increases in MCP-1 plasma levels because blood levels should fall after resection if the source of the elevated MCP-1 was the tumor. This study is interesting and I would like to give my suggestions to impact the authors understanding of the tumor tissue in the elucidation of aberrant molecular aspect changes in the tumor microenvironment and surgical margins to impact the paper. The authors should understand that rate of tumor recurrence following resection suggests that there are underlying molecular biometric changes in histologically normal tissue that go undetected by conventional diagnostic methods available that utilize contrast agents and immunohistochemistry. Current molecular technologies has the advanced specificity and sensitivity to monitor and identify molecular species indicative of these changes. These technologies (e.g. MALDI MS / IMS) indicate that the histologically normal tissue adjacent to the tumor expresses many of the molecular characteristics of the tumor. I recommend that the authors should visit and read this article (Oppenheimer SR, Mi D, Sanders ME, Caprioli RM. J Proteome Res 2010 May 7;9(5):2182-90) in order to improve the discussion of the paper. More suggestions and recommendation is that probably the results of this work and their impact to cancer research are discussed not fully, which I'll try to expound after I list some desired corrections:

General response: Thank you for the reference and your interesting suggestion that apparently normal tissues adjacent to a cancer are not in fact normal. This is a very interesting concept that deserves study and deep consideration. However, we do not think that it is necessary to invoke such a mechanism when trying to account for the development of distant metastases after resection of a primary tumor. We believe that the recurrences are the result of: 1) growth of very small already established (but not detected) metastases and/or 2) the establishment of tumor foci at distant locations from circulating viable cancer cells (that may have been shed at the time of surgery).

Comment 1: some abbreviations in the abstract are not decrypted, for example all of the PODs and CRC

Response: Manuscript is updated as per comment.

Comment 2: Abstract, lines 10 and 11: "median… was higher than…" By the means of statistics, not medians are being compared, but the samples itself. It can be said that levels differed, not medians.

Response: Manuscript is updated as per comment.

Comment 3: Introduction: should "in vitro" be typed in italics?

Response: Manuscript is updated as per comment.

Comment 4: Citation 9 seems to show that "chemotactic response is inhibited by MCP-1 monoclonal antibodies". Was that effect shown in vitro or in vivo? I think the results like that might mean much for this research.

Response: The citation 9 shows that the chemotaxis of endothelial cells is inhibited by MCP-1 antibodies in their in vitro and in vivo experiments. The manuscript has been updated.

Comment 5: I would highly recommend to carry out statistical analyses and describe them more accurately. For example, some of the variables are described as mean +/- SD, and others as median and CI, without even checking if the data is distributed normally or not. What is more, the comparison of MCP-1 levels for the Pre vs. Postoperative CRC is performed with the use of Wilcoxon signed rank test, which is the method of non-parametric statistics, and still the results are shown as Mean +/- SD, which is clear discrepancy of logic

Response: Statistical analysis was carried out as per your comments. Preoperative MCP-1 values in cancer and Benign populations were not normally distributed and as such their median values were appropriately compared using Mann and Whitney U test. The comparison of MCP-1 levels for the Pre vs. Postoperative CRC was performed with the use of Wilcoxon matched paired test and the data were reported as Median and CI values as suggested. The manuscript has been updated as per reviewer's comments.

Comment 6: It would be better if Figure 2 was first and Figure 1 be the second. It is unnecessary to speak on Figure 1 in the Methods section – this can be easily transferred to the Results section. Also, add the citation for Figure 1 to the 3.3 chapter in the results section.

Response: Your comments are well noted. However, the sentences that describe the collection of Benign PreOp blood were left in the methods section because it provides the reader with information as to when the plasma samples were obtained and how they were grouped and included in the study. Of note, the order of figure numbers in the manuscript is changed and their citations have also been updated.

Comment 7: The use of uppercase and lowercase letters in the word "preop" is different throughout the text. Please unify.

Response: Manuscript has been updated as per this comment.

Comment 8: The correlation values 0.2 to 0.4 are usually considered weak; is it competent to use these results for making any conclusions?

Response: The authors completely understand that the correlation demonstrated were weak (r_s0.2 to 0.4) despite the fact that the respective p values were significant ($p<0.05$). The manuscript has been updated to reflect this point.

Comment 9: Discussion: "blood levels were increased", "blood levels should fall" – it should be clarified.

Response: Manuscript has been updated as per this comment.

Comment 10: I believe that discussing the impossibility of obtaining enough blood samples is not the best ending for the paper. Probably it would be better to add some concluding paragraph to the end of the paper. NB: Overall, I think that it could be beneficial for the paper.

Response: Manuscript has been updated as per comment. A concluding paragraph is now included at the end of the revised manuscript.

"Conclusion:

At baseline, plasma MCP-1 levels are significantly elevated in colorectal cancer patients. Also, for at least 1 month after minimally invasive tumor resection, plasma MCP-1 levels are significantly elevated from the preoperative baseline. The early postoperative elevations (1st week) may be related to the acute inflammatory response associated with surgical trauma and anesthesia. Although unproven, it is believed that the elevations observed during weeks 2 through 4 are related to wound healing. MCP-1 joins the growing list of pro-angiogenic proteins whose blood levels are persistently elevated after colorectal resection (VEGF, PlGF, sVCAM, ANG-2, MMP-3, etc). These surgery-related plasma compositional changes may stimulate the growth of residual micrometastases early after resection. Further investigations are needed to determine the clinical ramifications, if any, of these transient yet significant changes. The search for and administration of anti-cancer agents that do not inhibit wound healing may be indicated.

Reviewer 2:

Comment 1: There are too many abbreviations. Especially it is very difficult to understand the summary section (especially preop, pts, postop, POD should be written in normal form, not in abbreviations).

Response: Manuscript was updated as per comment. Many of the abbreviations in the initial manuscript and abstract have been eliminated.

Comment 2: At page 4, "Weber et al. showed that the presence of a MCP-1 receptor antagonist or neutralizing MCP-1 antibody impaired the ability of ECs to migrate and close wounds, whereas the addition of MCP-1 facilitated repair." There should be a reference number for this explanation.

Response: The reference for this sentence has been added to the manuscript.

Comment 3: Introduction section is too long. Some part of it should be included in the discussion section.

Response: The manuscript was updated as per this comment. We have edited the introduction and moved some parts of it. However, the authors believe that it is important to explain to the surgical readership (who are not likely to have much knowledge of MCP-1) in the introduction what MCP-1 is and the physiologic effects that have been attributed to it. We also think it is important to explain in the introduction the rationale behind this line of research (the fact that similar persistent long duration protein elevations have been noted and that postop plasma has been shown to stimulate endothelial cell proliferation, migration and invasion [all critical to angiogenesis]) so that the reader will understand why

the study may have relevance. We apologize for the length of the introduction but hope it will be acceptable.

Comment 4: The authors should define the study period

Response: We have added to the manuscript the study period during which the patients in the study had their surgery (2003-2011).

"Consenting patients with CRC or benign colorectal disease (BEN) that underwent elective MICR during the period of 2003-2011 were identified from a larger population of patients who had been enrolled in an IRB-approved multicenter prospective data and blood banking protocol."

Comment 5: Exclusion or inclusion criteria should be defined more clearly.

Response: Manuscript is updated as per comment.

"The broadly stated purpose of this effort is to study the physiologic, immunologic, and oncologic ramifications of major abdominal surgery. Enrolled patients underwent surgery alone and did not receive a novel drug or other therapy. The indications and type of surgery as well as the demographic, operative, and short term recovery data was prospectively collected for all patients. Recently transfused patients, immunosuppressed patients (medication-related, HIV+, etc), and those who received radio- or chemotherapy within 6 weeks of surgery were excluded. Patients undergoing urgent or emergent surgery were, likewise, excluded".

Comment 6: The authors should define the adverse effects of high MCP 1 on timing of postoperative treatments more briefly with examples from current literatüre (if available)

Response: The manuscript is updated as per comment. After the section included below, the discussion goes on to discuss the possibility of administering anti-cancer agents during the month immediately following surgery and supports this idea with several references regarding the administration of agents given during this time window. One rationale for such treatment is that the month long blood compositional changes (MCP-1 as well as VEGF, ANG-2, etc) after surgery may stimulate the growth of residual mets.

". Persistently elevated levels of MCP-1 after MICR for CRC may promote recurrence in patients who harbor tumor micro foci. The complex process of residual tumor growth and metastasis may be supported by other angiogenic proteins whose blood levels remained elevated after MICR for CRC such as VEGF, PLGF, sVCAM-1 ANG2 and MMP3. There are case reports of rapid tumor growth and the development of metastases in cancer patients who undergo major surgery [23, 34].

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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