Author’s response to reviews

**Title:** Cytokine profiling reveals increased serum inflammatory cytokines in idiopathic choroidal neovascularization

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**Author’s response to reviews:**

Dear Prof. Lennikov and reviewers:

Re: Manuscript BOPH-D-19-00087R1, Authors: Shenchao Guo; Houfa Yin; Mingjie Zheng; Yizhen Tang; Bing Lu; Xinyi Chen; Qiuli Fu; Zhenwei Qin; Danni Lyu; Qiaomei Tang; Lifang Zhang; Jian Ma; Li Zhang; Xiaoyun Fang, M.D. “Cytokine profiling reveals increased serum inflammatory cytokines in idiopathic choroidal neovascularization”.

Sincerest thanks for your response and comments on our above manuscript. Those comments and suggestions are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. The manuscript has been carefully revised according to your suggestions which we hope meet with your approval. Revised portion is highlighted in red in the revised manuscript. The responds to the reviewers’ comments are listed below this letter.

I hope the revised version of the manuscript meets the requirements of your journal for publication. However, if you have more comments and questions or need more information, please don’t hesitate to contact us, and we will be happy to revise the manuscript again. Looking forward to hearing from you soon.

With best regards

Sincerely yours,
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Responds to the editor’s comments are as follows:

Editor Comments: Authors encourage to put extra attention to the the critical points raised by Dr. Mukwaya (Reviewer 2) regarding the statistical findings and conclusions of the manuscript (last for 4 points).

Response: Thank you very much for your comments. Your comments are very helpful for revising and improving our manuscript. We have revised relevant paragraphs (Line 120-121, page 6; Line 212-214, page 10) and explained our decision in detail.

Responds to the reviewers’ comments are as follows:

Reviewer comments:
Ebru Nevin Cetin, Associate professor, MD, FEBO, FICO (Reviewer 1):

1-The age and gender information can easily be given in the text, so table 1 is not necessary.

Response: Thank you very much for your comments. Your comments are very helpful for revising and improving our manuscript. We have removed Table 1 from the manuscript and have placed this information in the main text (Line 126-131, page 6).

2-Please provide the definitions for the abbreviations in the tables.

Response: Thank you very much for your suggestions. Your rigorous attitude to scientific research is appreciated. We have added the definitions for the abbreviations in the tables.

3-The investigators reported that they had found significantly increased intraocular levels of several cytokines in the aqueous humor of eyes with ICNV (ref.17). Perhaps they could discuss more about what this new study adds to what is already known about idiopathic choroidal neovascularization and inflammation. Giovannini et al. reported that the use of systemic steroid before photodynamic therapy has shown better BCVA outcome than photodynamic therapy alone, reducing the mean number of photodynamic therapy applications (BJO 2007). What do the authors think about adding anti-inflammatory treatment to anti-VEGF treatment regime in such group of patients? Please discuss.

Response: Thank you very much for your suggestions. Your suggestions are very helpful for revising and improving our manuscript. We have revised relevant paragraphs (Line 190-198, page 9).

Anthony Mukwaya, Ph.D. (Reviewer 2):

Abstract

Lines 24-26, I guess what the authors are referring to as the background is instead the objective of their study. If so, please revise accordingly.

Response: Thank you very much for your comments. Your comments are very helpful for revising and improving our manuscript. We have revised relevant paragraphs (Line 24-28, page 2).

Lines 30-32. I wonder if basic fibroblast growth factor, granulocyte-macrophage colony-stimulating factor and vascular endothelial growth factor, all fall under the classification of cytokines.
Response: Thank you very much for your comments. Your rigorous attitude to scientific research is appreciated. According with previous studies, basic fibroblast growth factor, granulocyte-macrophage colony-stimulating factor and vascular endothelial growth factor fall under the classification of cytokines[1, 2].

Background

Lines 61-66, authors state that the expression profile of inflammatory cytokines in AMD where shown to increase in advanced AMD. What is the difference in the pathophysiology between AMD and ICNV which motivates the authors to conduct the current study? If any, this should be spelt out clearly in their introduction.

Response: Thank you very much for your suggestions. Your rigorous attitude to scientific research is appreciated. AMD is defined as a degenerative disorder in persons ≥50 years of age[3]. The advanced form of AMD can be divided into neovascular and non-neovascular atrophic types, and CNV is a clinical hallmark of neovascular AMD. ICNV is defined as CNV occurring in patients younger than 50 years with no detectable primary ocular or systemic diseases, such as pathologic myopia, angioid streak, trauma, or other inflammatory or hereditary disorders[4]. Although extensive research has been carried out to investigate the basic and clinical aspects of AMD and ICNV, the pathogenesis remains unknown[5].

Methods

Lines 77-79. Please state the number of patients recruited

Response: Thank you very much for your suggestions. Your suggestions are very helpful for revising and improving our manuscript. We have revised relevant paragraphs (Line 77, page 4).

Lines 77-79. What is the meaning of naïve ICNV? This is not mentioned anywhere in the background.

Response: Thank you very much for your comments. Your comments are very helpful for revising and improving our manuscript. Naïve ICNV was determined as newly diagnosed active ICNV without any previous treatment. We have revised relevant paragraphs (Line 79-80, page 4).
Lines 79-83. Why was it that only patients undergoing first-time ranibizumab treatment were considered in the study, and not any other treatment?

Response: Thank you very much for your comments. Your rigorous attitude to scientific research is appreciated. Nowadays, intravitreal ranibizumab treatment is efficacy and safety in ICNV, and superior to PDT treatment[4]. Therefore, ICNV patients received intravitreal ranibizumab treatment in our study. In addition, a significant systemic upregulation of proangiogenic cytokine PIGF was observed after intravitreal anti-VEGF treatment, therefore only patients undergoing first-time ranibizumab treatment were considered in our study.

Lines 98-102. Why were 32 controls recruited into the study and not the same number as the patients in the study population?

Response: Thank you very much for your comments. Your rigorous attitude to scientific research is appreciated. As smoking is a known cause of raised systemic inflammation and risk factor for CNV development, we excluded 2 smoking controls among the 32 controls recruited into the study. So the controls were not the same number as the patients in the study population.

Was age a factor for the recruitment of the controls? If so, please state clearly the cutoff age for inclusion.

Response: Thank you very much for your comments. Your comments are very helpful for revising and improving our manuscript. Indeed, age was a factor for the recruitment of the controls in our study. Healthy volunteers less than 50 years old were recruited as a control group in our study. We have revised relevant paragraphs (Line 101, page 5).

How was "no evidence of active ocular or systemic disease" determined in the controls prior to recruitment into the study?

Response: Thank you very much for your comments. Your rigorous attitude to scientific research is appreciated. Healthy volunteers with no evidence of active ocular or systemic disease were confirmed by clinical anamnesis, examination, and blood analysis. We have revised relevant paragraphs (Line 101-103, page 5).

Lines 116. Clarify when each of the statistical tests were performed.
Response: Thank you very much for your comments. Your rigorous attitude to scientific research is appreciated. We have revised relevant paragraphs (Line 120-121, page 6).

Results

Authors find a positive correlation between GM-CSF and CRT, however, the expression level of GM-CSF was not different between the patients and control (p value 0.394). Therefore, how reliable is the correlation analysis finding for this factor?

Response: Thank you very much for your comments. Your rigorous attitude to scientific research is appreciated. Difference detection and correlation analysis are two independent outcomes. Although the expression level of GM-CSF was not different between the patients and controls (P = 0.394), however, we came to a finding that the GM-CSF levels in serum were positively related to CRT (r = 0.519, P = 0.002), P<0.05 means the result is statistically significant and statistically reliable.

Discussion

Lines 149-154. Authors state that only a limited number of systemic cytokines have been assayed for in the past in ICNV, however, in the study they refer to by Yang et al. a total of 11 factors were investigated compared to the 7 assayed for here. Authors should therefore provide a better reason for conducting their study.

Response: Thank you very much for your comments. Your rigorous attitude to scientific research is appreciated. Yang et al. investigated 3 inflammatory cytokines in the serum of ICNV patients, however, we analysed 7 inflammatory cytokines, which were found to be significantly increased in the aqueous humour of ICNV patients in our previous study. The purpose of this study was try to investigate serum cytokine profiles in ICNV[6, 7].

Conclusion

According to the findings of the authors, not both IL-17 and GM-CSF correlated with lesion size. Please clarify this text and be consistent throughout.

Response: Thank you very much for your suggestions. Your suggestions are very helpful for revising and improving our manuscript. GM-CSF was not correlated with lesion size. We have revised relevant paragraphs (Line 212-214, page 10). We are very sorry for this mistake and implore your forgiveness.
The conclusion that "ICNV may be associated with high levels of serum inflammatory cytokines" may be an over statement, given that only 2/7 (28%) of the analyzed factors showed a correlation of some sort with ICNV, and of the two factors, GM-CSF was not significantly different between the study population and the controls (p= 0.394).

Response: Thank you very much for your comments. Your comments are very helpful for revising and improving our manuscript. We have revised relevant paragraphs (Line 212-214, page 10).

Reference


