Author’s response to reviews

Title: Hyperreflective foci in OCT image as a biomarker of poor prognosis in diabetic macular edema patients treating with Conbercept in China

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Author’s response to reviews:

David Antonetti (Reviewer #1)

1. Abstract. Please include p values of HF decrease in the abstract.

   According to the reviewer’s constructive suggestion, we added the p value of HF decrease: p=0.0045, p < 0.0001 and p=0.0045, respectively.

   Please see line 42 in the abstract section.

2. Abstract. Please expand on "The final BCVA was associated with baseline HF (provide statistics, etc.), since this is the main outcome of the study.

   We appreciate the reviewer’s suggestion. According to the reviewer’s constructive suggestion, we added “Correlation analysis showed that there was a positive significant correlation between the baseline number of HFs in the inner retina, outer retina, subretina and final BCVA (r=0.571, p=0.002; r=0.464, p=0.017; r=0.405, p=0.04 respectively). There was also a significant positive correlation between outer retinal HFs reduction, total retinal HFs reduction and increase of BCVA (r=0.40, p=0.043 and r=0.393, p=0.04 respectively).” in the abstract.

   Please see line 49-58 in page 1 of abstract section and line 1 in the page 2 of abstract section.

3. Awkward wording in line 17.
According to the reviewer’s constructive suggestion, we changed the sentence to: Similar to aflibercept, conbercept, with a molecular weight of 143kDa, consisting of the binding domains of VEGF receptor 1 and VEGF receptor 2 fused to the Fc portion of human immunoglobulin G1.

Please see line 18-24 in the introduction section.

4. Line 42 please describe loading dose frequency here (monthly)?

We appreciate the reviewer’s suggestion. The loading dose frequency is monthly injection for 3 months. We added the information in the manuscript.

Please see line 43 in the introduction section.

5. Line 48 specify if retinal or vitreous HF. If vitreous is not used, why not?

We are sorry for the unclear description. The HFs mentioned in this study were all retinal HFs. We added this information in the manuscript. As retinal HFs have been proposed to be precursors of hard exudates, migrating RPE cells, degenerated photoreceptor cells, or aggregations of activated immune cells, such as microglia (Ophthalmology. 2009; 116(5):914–20. Invest Ophthalmol Vis Sci. 2012; 53(9):5814–8. Ophthalmology. 2010; 117(10):1996–2002 Am J Ophthalmol. 2012; 153(4):710–7, 7.e1), we chose to investigate retinal HFs in this study.

Please see line 50 in the introduction section.

6. Methods: Concerns: You state this was a retrospective study, so it is non-randomized. Lines 15-28 state "Patients chose" but this does not adequately explain risk/benefit/alternative discussion, and the decision is not solely with the patient but with the doctor's guidance as well. Selection bias may be large and unaddressed here. You mentioned later on that the patients needed to pay large amounts of money out of pocket, but not the bias that this may bring.

We are sorry for the unclear description. All the DME patients were offered 3 treatment choices: grid laser, Lucentis and Conbercept. We explained in detail how these treatment works and the risk and benefits the patients would get. The grid laser treatment was covered by medical insurance and the others two was not. Lucentis costed 1000 USD and Conbercept costed 700 USD for each injection. As this was a retrospective study, there were many selection bias including the treatment chosen by the patients. We added these information in the methods section discussion section.
Please see line 27-33 in the page 1 of methods section, and line 5-7 in the page 3 of discussion section.

7. Line 39- define severe cataract

We are sorry for the unclear description. We excluded all the patients who had lens opacity in slit lamp examination. We changed it to “cataract” in the manuscript.

Please see line 53 in the methods section.

8. Line 42 - What was the foveal exclusion zone size criteria if any?

We are sorry for the unclear description. Any retinal disease that included the central 1 mm of the fovea were excluded in this study.


We are sorry for the unclear description. All the patients continued follow-up in our clinic. Nine of them showed a recurrent DME after the first 3 injections. Among them, 3 received another conbercept injection. The other 6 refused further anti-VEGF treatment due to financial reason. They were treated with grid laser afterwards.

10. Results : Line 59: 77% males seems off, please comment on why there was a sex bias.

We appreciate the reviewer’s comments. This was a retrospective study, although many DME patients were treated with conbercept at our clinic, the OCT images that met the criteria for analysis were limited. That’s the reason why the number of included patients was small. It can also explain the patients bias in this study to some extent.

11. Page 2 Line 14: what were they treated with? Observation? Topicals?

We appreciate the reviewer’s comments. They did not receive anti-VEGF injection until they were referred to our clinic. They were usually treated with topical NSAIDs, traditional Chinese medicine or just observation.
12. Page 3 line 17 provide statistics to support this claim of decreased HF before and after treatment.

We appreciate the reviewer’s comments. The detailed statistics was showed in the manuscript: “The number of HFs was 5.4±4.2 in inner retina, and 5.2±5.2 in outer retina. There was no statistical difference concerning HFs numbers between these two layers. However, the number of HFs was only 0.88±1.9 in the subretinal space, which was much lower than that of inner and outer retina (p=0.0004 and p=0.0008 respectively).”

13. Line 31: you state "significant correlation" between final BCVA and baseline HFs, but r values are small (~.5 and ~.4) showing only mild correlation. Especially since this is your main point of this paper. Line 42: same comment as above.

We are sorry for the misunderstanding. “Significant” was used to state that the p value was minor than 0.05. We did not discuss the r values in this manuscript previously, and we added these information in the discussion section.

Please see line 48-54 in page 2 of the discussion section.

14. Discussion: Line 39- I don't know how predictive this is with the given r values

We appreciate the reviewer’s comments. The results showed that there was a positive significant correlation between the baseline number of HFs in the inner retina, outer retina, subretina and final BCVA (r=0.571, p=0.002; r=0.464, p=0.017; r=0.405, p=0.04 respectively). There was also a significant positive correlation between outer retinal HFs reduction, total retinal HFs reduction and increase of BCVA (r=0.40, p=0.043 and r=0.393, p=0.04 respectively). As the r values in the correlation analysis were moderate, we changed the sentence to: “Moreover, the number of HFs may be a predictive factor of poor final BCVA in DME patients.”

Please see line 10-13 in page 1 of the discussion section.

15. Page 2 Line 23 - AntiVEGF medications do not address production of VEGF, they bind VEGF

We appreciate the reviewer’s comments. We changed the sentence to: “Anti-VEGF agents have been found to reduce hyperpermeability through binding VEGF.”

Please see line 56-59 in page 1 of discussion section.
16. Line 42: significantly overstated. You have not shown proof that the blood retinal barrier is "alleviated"

We appreciate the reviewer’s comments. We changed the sentence to: “As Conbercept has been found to inhibit the breakdown of blood-retinal barrier in diabetic rats (Diabetes, obesity & metabolism 2012, 14(7):644-653.), we speculate that it may alleviate the damage of blood-retinal barrier in DME patients, and the underlying mechanism need to be investigated further.”

Please see line 14-23 in the page 2 of discussion section.

17. Page 3 Line 3-7, do you mean "correlates with" instead of "indicate", please address if you mean to say that this is a predictive factor or a correlation

We are sorry for the inappropriate writing. We changed “indicate” to “had a moderate correlation with”.

Please see line 48-54 in page 2 of the discussion section.

18. Line 20- was baseline HF counts correlated with CMT?

We appreciate the reviewer’s comments. There was no significant correlation between baseline HFs and CMT.

19. The authors claim the hyperreflective foci (HF) may act as a biomarker for poor visual outcomes, although this was clearly not demonstrated. The authors do attempt to provide data for HF as a clinical correlate to edema and visual acuity in diabetes. However, even here, there is a limited study size and only a moderate correlation r values of baseline HF as compared to final BCVA (~.4, ~.5). Further, the baseline HF to final BCVA does not really make sense. What is needed is a correlation between the change in HF and change in BCVA after treatment. Importantly, the HF changes over drug treatment are not plotted.

We appreciate the reviewer’s comments. In addition to the correlation analysis between baseline HFs and final BCVA, we also did the correlation analysis between the changes of HFs and the changes of BCVA in the results section: “There was also a significant positive correlation between outer retinal HFs reduction, total retinal HFs reduction and increase of BCVA (r=0.40, p=0.043 and r=0.393, p=0.04 respectively)”. Furthermore, the changes of HFs after anti-VEGF treatment were listed in the result section: “4. Changes of HFs before and after anti-vascular endothelial growth factor treatment”.
20. The authors fail to mention significant selection biases associated with their study design, sex biased patient demographics, and the out of pocket cost associated with this medication influencing selection of which AntiVEGF medication to use.

    We appreciate the reviewer’s comments. As this was a retrospective study, selection bias could no be avoided. We added the information in the discussion section.

    Please see line 4-7, 15-18 in the page 3 of discussion section.

Osman ÇEKIÇ (Reviewer 2)

1. Please add the name of post hoc test applied following ANOVA.

    We thank the reviewer for the suggestion. We used Tukey’s test in the post hoc test following ANOVA. We added it in the “methods” section.

    Please see line 4 in the page 3 of methods section.

2. Please clarify whether you used SEM or SD after mean values.

    We appreciate the reviewer’s suggestion. We used SD in the manuscript, and we added the information in the “methods” section.

    Please see line 57 in the page 2 of methods section.