Author’s response to reviews

Title: Reduced Frequency of Intravitreal Methotrexate Lowers the Risk of Keratopathy in Vitreoretinal Lymphoma Patients

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Yoshihiko Usui (Reviewer 1): Overall, the manuscript is clearly written and represents some contribution to the field. Although the methodology was apparently properly used and I agree with the potential value of this work, the limited sample size diminish my enthusiasm for this work.

And, I also agree with that the optimal treatment for intraocular lymphoma is not yet determined.

Coupled with the disappointingly low number of subjects, it is highly likely that your findings are spurious.

1. The authors should change intraocular lymphoma to vitreoretinal lymphoma.
Reply: We have changed intraocular lymphoma to vitreoretinal lymphoma as advised.
2. What is the study type? Looks like case-control or comparative method evaluation, but this is never explicitly stated.
Reply: This is a real world study. Treatment methods depended on the systemic conditions of the patients. It is not randomized.

3. How many were rejected, how many were excluded?
Reply: All PCNSL patients with intraocular involvement were included in the study. No one was rejected. Two patients (21, 22) were excluded from comparation of treatment outcomes. They did not receive intravitreal methotrexate because of progression of intracranial lesions.

4. How many patients have been done radiation before intravitreal MTX injection. Radiation higher the risk of keratopathy in vitreoretinal lymphoma patients.
Reply: A total of 5 patients were treated with WBRT (whole brain radiotherapy). One patient received WBRT before intravitreal MTX injections, and the remaining received WBRT due to the progression or recurrence of intracranial lesions during intravitreal MTX injections. None of the patients received ocular radiation. From literature review and clinical experiences, The most common complication of WBRT is delayed neurotoxicity[1]. Keratopathy is more likely to correlate with ocular radiation. We did not perform ocular radiation in this study.

5. Why are the groups not of equal size? The manuscript is not clear here.
Reply: This is a real world study, not randomized controlled trial. Treatment methods depended on the systemic conditions of the patients in the two groups. This study is a description of our preliminary observations.

Shahar Frenkel (Reviewer 2): Dr. Zhou and colleagues tested what most bothers patients treated with IVit MTX for VRL - the keratopathy. Keratopathy is the reason why some clinicians give only partial treatment, see recurrences and erroneously decide that IVit MTX is not good for their patients. As such, this is an important paper. The manuscript is written well and clear. Please see specific comments.

Background:
1. VRL does NOT involve the uveal tract.
Reply: We have deleted it as advised.

2. Brackets for the references should be spaced from the text.
Reply: we have added space before the references.

3. Please be consistent - the proper term is vitreo-retinal lymphoma VRL. Intraocular lymphoma also includes to choroidal lymphoma, which is a different entity altogether.
Reply: We have changed intraocular lymphoma to vitreoretinal lymphoma as advised.

4. Some articles are missing: "…antibody) are currently THE main treatment…". "Despite THE efforts, intraocular lymphoma…"
Reply: We have added THE.
5. This last sentence is also wrong in context in addition to the bad grammar: VRL has a good prognosis. It is the CNS component that entails a bad prognosis. Treatment of the VRL portion does not change the CNS outcome.
Reply: We have revised the statement.
Vitreoretinal lymphoma alone has a good prognosis. However, the prognosis of central nervous system lymphoma remains poor.

M&M:

6. Diagnosis: The only missing component in this fine description is the lack of PCR analysis of the specimens. I would appreciate a discussion of its absence.
Reply: Do you mean IgH rearrangement or MYD88?
For MYD88, we have stated in this article. 75% showed positive. If needed, we can add it to the article.
For IgH rearrangement, it is a good method for the diagnosis of VRL. We have tried to use this test to make the diagnosis more accurate. However, it failed, no matter we sent the specimens to the laboratory in our hospital or to the commercial company. Another reason we did not perform this test is that the positive rate of the cytological examination in the study is 85%. It is higher than most studies published. One pathologist in our hospital improved the centrifuge. It was easier to collect the vitreous cells.

7. Treatment: Here lies a great flaw: it appears that radiation treatment could have been added. This messes the results, as one would expect to see the results of IVit MTX alone in a study like this.
Reply: We did not perform ocular radiation. Only patients treated with IVit MTX were included in this study. Five patients were treated with WBRT (whole brain radiotherapy), not ocular radiation.

Results:

8. Please also add a Snellen equivalent next to each LogMAR result.
Reply: The Snellen equivalent are added as advised.

9. Table 2: did WBRT include the eyes in the radiation fields? If so, these patients should be excluded from this study.
Reply: WBRT did not include the eyes in the radiation fields. None of the patients received ocular radiation in this study.

10. Fig. 3 is far from depicting a typical IVit.MTX-type of keratopathy. It is far too gentle.
Reply: It is an authentic photo taken from a patient when he was observed to have keratopathy the first time. Actually, we do not have professional anterior segment camera in our hospital. All photos were taken by smart phones through slit lamp, often with poor quality. This picture is actually the best we have. If not appropriate, can we remove it from the article?
Discussion:

11. It appears that the authors follow the recommendation that any CNSL patient undergo routine ophthalmic evaluation repeatedly, and not just at diagnosis of CNSL. This should be stated clearly.
Reply: We have revised the manuscript.
Complete and repeated ocular evaluation is suggested for PCNSL patients.

12. The name of the entity is vitre-RETINAL lymphoma. However, it appears that hardly any of the patients had retinal involvement. This is an important observation to state.
Reply: We describe the retinal abnormalities in 6 eyes briefly. To complement the description, we reviewed the OCT findings of the patients in this study and added it to the article.

Abnormal manifestations of the retina were recognized in 6 eyes, including hemorrhage and exudation (4 eyes), retinal atrophy (1 eye) and creamy white retinal lesions (1 eye).
Fifteen eyes showed abnormalities on OCT, including outer retina fuzzy borders (10 eyes), subretinal deposits (5 eyes), retinal pigment epithelium detachment from Bruch’s membrane (4 eyes), epiretinal membrane (1 eye) and outer retina atrophy (1 eye).

13. I completely disagree with the authors' cautionary statement on minimizing treatment for patients without CNS disease, as previous publications did not find any effect of the IVit injections on the CNS portion of the disease.
Reply: We have deleted this statement.

Yo-Chen Chang (Reviewer 3):

1. Page 1 (title page): Author's first and last names, some are separated, some are linked together, please correct
Reply: We have revised as advised.

2. Page 2, line 12: I suggest that "divided" to be replaced by "assigned"
Reply: We have revised as advised.

3. Page 4, line 4: "intraocular lymphoma is also known as", the "intraocular" should be "Intraocular".
Reply: We have revised as advised.

4. Page 4, line 15: "intraocular lymphoma is even responsive to", the "intraocular" should be "Intraocular". And the "even" might be replaced by "initially".
Reply: We have revised as advised.

5. Page 4, line 21: The "ophthalmologist" should be "ophthalmologists"
Reply: We have revised as advised.

6. Page 5, line 2-5: Please cite references for "The most widely used intravitreal methotrexate treatment regimen consists of three phases: induction, consolidation and maintenance. In detail, it is composed of injection with a dose of 400μg/0.1 ml twice a week for the first four weeks, weekly for the followed eight weeks, and then monthly for the last nine months."

7. Page 5, line 2-5: "intraocular lymphoma prognosis" might be replaced by "the prognosis of intraocular lymphoma".
Reply: We have revised as advised.

8. Page 5, method part, line 4: "Patients with systemic lymphoma were excluded". Do you mean "Patients with pre-existing systemic lymphoma were excluded"?
Reply: We have deleted this ambiguous sentence. Actually, every patient in the study had PCNSL. The term PRIMARY already excludes patients with systemic lymphoma before CNS involvement.

9. Page 6, line 4: "diagnostic 25G-pars plana vitrectomy (PPV) which were performed by one skilled surgeon." Please add the initials of the surgeon.
Reply: We have added the name of the surgeon.

10. Page 6, treatment part, line 4: "6 patients (8 eyes) treated with induction-maintenance regimen", the "6" might be replaced by "Six"
Reply: We have revised as advised.

11. Page 6, treatment part, line 10-11 "Complete Remission was described as the absence of malignant cells in vitreous, retina or optic nerve head." How do the authors confirm the complete remission? By repeated PPV, etc?
Reply: Our presentation is not precise. "Complete remission" might be replaced by "Complete clinical remission". By reviewing references concerned and clinical experience, the criteria are as follows: Complete clinical remission was described as the absence of obvious tumor lesions in the vitreous, retina or optic nerve head by slit lamp examination, B-ultrasound scan and OCT examination.

12. Page 8, line 3-4: "However, 38% of patients diagnosed with intraocular lymphoma had no ocular symptoms." Actually, from literature review and clinical experience, most patients have painless decrease in vision or floaters. Please explain why so many patients in your series had no symptoms?
Reply: The proportion of symptomatic patients was relatively low. This may be because all PCNSL patients, with or without ocular symptoms, underwent ophthalmic examinations to determine the presence of vitreoretinal lymphoma. We have added the above sentence in the DISCUSSION part. Some asymptomatic patients in the study were diagnosed with VRL during routine ophthalmic evaluation.

13. Page 8, line 18-20: "The undiluted vitreous specimens contained 121×10^6 cells/mL compared with 17×10^6 cells/mL in diluted samples (P=0.009)." Are the two data represented the mean of the 33 eyes? If they are, please add "mean" or "average" and give the data as Mean±STD.
Reply: The two data represent the mean of the 33 eyes. We have revised the manuscript.
Page 9, line 11-12: "12 eyes did not complete the entire treatment but achieved ocular clinical remission." Please use "Twelve" instead of "12". Please mention the number in both group separately.
Reply: We have revised as advised.

Page 10, line 11-12: "However, 9 patients suffered recurrence of the CNS lymphoma, 6 patients in Group A and 3 in Group B (P=0.666)." The sentence is strange, please rewrite.
Reply: We have revised the sentence.

Page 12, last paragraph, line 3-4: "The lower incidence of keratopathy in Group B is probably due to the shorter injection duration of high frequency." I remember the protocol in Group B is longer injection duration of low frequency, am I right?
Reply: We have revised the ambiguous statement. Shorter injection duration should be replaced by increased injection interval.

Page 13, The paragraph: "Rituximab is a mouse-human chimeric monoclonal antibody against the CD20 antigen on the membrane of B cells(24, 30, 33). Some nonrandomized studies showed that intravitreal rituximab monotherapy might be effective for intraocular lymphoma(28, 33, 34). Long-term and large sample studies of the rituximab treatment are still absent. More evidence is needed to support Rituximab treatment as a first-line treatment." Since the authors did not use Rituximab, I suggest delete the paragraph.
Reply: We have revised as advised. The paragraph is deleted.

Generally speaking, the content of the manuscript is interesting, however, major revision and professional language editing by native English speaker are necessary.
Reply: The manuscript has been edited by native English speaker.