PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Comprehensive analysis of vitreous specimens for uveitis classification: A prospective multicentre observational study
AUTHORS	Maruyama, Kazuichi; Inaba, Tohru; Sugita, Sunao; Ichinohasama, Ryo; Nagata, Kenji; Kinoshita, Shigeru; Mochizuki, Manabu; Nakazawa, Toru

VERSION 1 – REVIEW

REVIEWER	Erika Marie Damato Birmingham and Midland Eye Centre Sandwell and West Birmingham NHS Trust Dudley Road Birmingham UK
REVIEW RETURNED	08-Nov-2016

GENERAL COMMENTS	The study has several limitations, which should be discussed. 1. the authors have divided the samples into patients with sarcoidosis, suspected sarcoidosis, cancer, viral infection, non-sarcoidosis and other. What is the reason or rationale for this division? 2. No details are given regarding treatments. It may be considered that systemic immunosuppression, systemic steroid or local steroid may influence the cellular composition of the vitreous and may affect the results of this study. 3. There are no patients with TB included in this study. Presumed ocular TB is a major differential for sarcoidosis in the clinical environment and is also a granulomatous uveitis. Are the authors sure that similar CD4:CD8 results could not occur in the presence of ocular TB? 4. In the “other” group, patients with endophthalmitis are included. Normally vitrectomy is carried out only in fulminant cases, where the vision is poor. Does this group contain low grade, indolent, post operative, fungal or fulminant endophthalmitis? 5. The “other” group is heterogeneous and it could be argued that behcet patients have a very different cellular infiltrate compared with toxoplasmosis. The heterogeneity could also confound the results, as this group will be less likely to have a characteristic vitreous profile when compared to the homogenous sarcoidosis group. 6. There is a spelling error in the table – non-sarcoicosis should be non-sarcoidosis. 7. The characteristic findings in patients with sarcoid/ lymphoma / viral infection are interesting and the authors provide a convincing argument for the value of such profiling.

REVIEWER	Burak Turgut Fırat University/TURKEY
REVIEW RETURNED	24-Jan-2017

GENERAL COMMENTS	In abstract: Please correct and be sure the Design of the study: Prospective observational case series?Interventional? In Introduction: Page 5 line 41. please add "In our previous study,"to the beginning "We showed vitreous....) In Methods: Please clarify whether or not aqueous samples were obtained. It seems the aqueous samples were taken for PCR. If so, please detail it and give the results of PCR in the results section Page 6 line 10. Please delete "experimental" and correct as "invasive and therapeutic" Page 7 line 7-15, in Table, please provide the number of cases/eyes with diagnosed sarcoidosis In results: Page 11, line 37-57, the data on CD8 is confusing. Please clarify and improve the presentation of data. In Discussion: Discussion is too confusing. I suggest that authors must rewritten the Discussion section and enlarge it The results should be discussed in detail in this section In references: References should be increased and updated In Legends: Figure 1 legend should be summarized

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Erika Marie Damato
Institution and Country: Birmingham and Midland Eye Centre, Sandwell and West Birmingham NHS Trust, Birmingham, UK
Competing Interests: none declared

Thank you for inviting me to review this paper.

The authors report the findings of a study investigating vitrectomy specimens from patients with uveitis.

The study has several limitations, which should be discussed.

1. The authors have divided the samples into patients with sarcoidosis, suspected sarcoidosis, cancer, viral infection, non-sarcoidosis and other. What is the reason or rationale for this division?

Thank you for the important question. The reason for this division was that the treatment methods were different. Most sarcoidosis patients are treated with steroids, but uveitis caused by other infectious diseases, lymphoma, or with another non-infectious aetiology are treated with other drugs, such as MTX, cyclosporine, or biological drugs. Moreover, the number of patients with each type of uveitis differed at our outpatient clinic, with sarcoidosis patients being the most frequently seen. Therefore, our division of the samples was based on differing clinical conditions.

2. No details are given regarding treatments. It may be considered that systemic immunosuppression, systemic steroid or local steroid may influence the cellular composition of the vitreous and may affect the results of this study.

Thank you for the comment, with which we agree. Indeed, most of our subjects received systemic or local treatment before surgery. As we mentioned in the paper, the patients who received surgery had already experienced various complications, such as macular oedema, preretinal membrane and severe vitreous opacity. These complications would have affected visual function if treatment had not been administered, especially in the patients with infectious endophthalmitis. As you mentioned, systemic immunosuppression may have affected the cellular composition of the vitreous and thus the results of the present study. We cannot argue against this point. However, even in the sarcoidosis patients who received non-immunosuppressive treatments, we did not see any difference in cellular composition between the treatment and non-treatment groups in previous study (Medicine 2016 Dec; 95). We have added an explanation of the effects of immunosuppressive treatment to the manuscript at discussion section.

3. There are no patients with TB included in this study. Presumed ocular TB is a major differential for sarcoidosis in the clinical environment and is also a granulomatous uveitis. Are the authors sure that similar CD4:CD8 results could not occur in the presence of ocular TB?

Thank you for this interesting question. Indeed, we would have very much liked to collect vitreous samples from patients with tuberculosis. However, ocular TB patients needing surgical treatment are very rare in Japan. Therefore, we could not include any such patients in the present study. In the future, if we have the chance, we will be very interested in collecting this type of vitreous sample and measure the CD4/CD8 ratio.

4. In the “other” group, patients with endophthalmitis are included. Normally vitrectomy is carried out only in fulminant cases, where the vision is poor. Does this group contain low grade, indolent, post operative, fungal or fulminant endophthalmitis?

Thank you for your comment. The endophthalmitis patients (n = 10) in the present study included 7 with low- or middle-grade bacterial infection, 2 with mild fungal infection and 1 with postoperative endophthalmitis. As you mentioned, it would be dangerous to collect samples from patients with high-grade endophthalmitis. Therefore, we did not collect any samples for flow cytometry from patients with severe endophthalmitis.

In response to your comment, we added the following passage to the discussion section: “However, it is dangerous to collect samples from patients with high-grade endophthalmitis, due to the presence of severe vitreous opacity. Therefore, we only collected vitreous samples from patients with low- or middle-grade endophthalmitis.”

5. The “other” group is heterogeneous and it could be argued that behçet patients have a very different cellular infiltrate compared with toxoplasmosis. The heterogeneity could also confound the

results, as this group will be less likely to have a characteristic vitreous profile when compared to the homogenous sarcoidosis group.

Thank you for this comment, with which we agree. As you mentioned, our results indicated that the 2 Behçet disease patients had low CD4/CD8 ratios (i.e., less than 1.0) while the 2 toxoplasmosis patients had differing (high and low) CD4/CD8 ratios. Therefore, the cellular infiltrates were indeed very different in the 2 groups. When compared to groups with larger numbers of patients, such as the sarcoidosis, lymphoma and viral infection groups, we conclude that the non-sarcoidosis group included a low number of diagnostic diseases. However, when compared to the sarcoidosis patients, only 3 patients in the non-sarcoidosis group had a high CD4/CD8 ratio. Therefore, we consider that the heterogeneity of the non-sarcoidosis group should not have affected our results or conclusions. In the future, if we can collect more samples and identify a higher number of diagnostic diseases, we would like to repeat this comparison.

6. There is a spelling error in the table – non-sarcoicosis should be non-sarcoidosis.

Thank you for your comment. We have corrected the mistake.

7. The characteristic findings in patients with sarcoid/lymphoma/ viral infection are interesting and the authors provide a convincing argument for the value of such profiling.

Thank you your comment. We would like to see this system used to diagnose uveitis in a general clinical setting in the near future.

Reviewer: 2
Reviewer Name: Burak Turgut
Institution and Country: Fırat University/TURKEY
Competing Interests: None

In Abstract:
Please correct and be sure the Design of the study: Prospective observational case series? Interventional?

Thank you for your question. This was a prospective observational case study. We have added this detail to the description of the study design in the abstract.

In Introduction:
Page 5 line 41. Please add "In our previous study" to the beginning "We showed vitreous....)

Thank you for your comment. We have revised this sentence.

In Methods:
Please clarify whether or not aqueous samples were obtained. It seems the aqueous samples were taken for PCR. If so, please detail it and give the results of PCR in the results section.

Thank you for your comment. We did not perform aqueous humour sample collection in the present study. We have omitted this information.

Page 6, line 10. Please delete "experimental" and correct as "invasive and therapeutic".

Thank you for your comment. We have revised this sentence accordingly.

Page 7, line 7–15, in Table, please provide the number of cases/eyes with diagnosed sarcoidosis.

Thank you for your comment. We have added the number of cases/eyes (46/61) to the table.

In Results:
Page 11, line 37–57, the data on CD8 is confusing. Please clarify and improve the presentation of data.

Thank you for your comment. We have attempted to clarify and improve the presentation of the data by revising the manuscript as follows: 'The CD8+ population of lymphocytes was significantly higher in the vitreous samples obtained from patients with viral infection ($P < 0.05$) and intraocular tumour/PIOL than in the samples from patients with definitive sarcoidosis, suspected sarcoidosis and uveitis of unknown aetiology (Figure 1c). However, we did not find any significant differences between the intraocular tumour and non-sarcoidosis patients, or between the viral infection and non-sarcoidosis patients (Figure 1c)'.

In Discussion:
Discussion is too confusing. I suggest that authors must rewritten the Discussion section and enlarge it. The results should be discussed in detail in this section.

Thank you for your comment. We have done our best to clarify and expand this section. Moreover, we have discussed the results in more detail in the revised version.

In References:
References should be increased and updated.

We have increased the number of references and updated them.

In Legends:
Figure 1 legend should be summarized.