Peer Review File

Article information: https://dx.doi.org/10.21037/aes-21-59

Reviewer A

Comments:

The authors present a generally well-written review of the diagnosis and management of intraocular lymphoma, which is a challenging task given the complexity of this disease. Several revisions are suggested, the majority of which are relatively minor. There are, however, several major revisions to be made, mostly pertaining to Figure 5:

Comment 1: First of all, where are the A and B panels referred to in the legend? There does not seem to be any fundus photo or autofluorescence photo shown. Please add.

Reply 1: Figure 5A and 5B were unfortunately unable to be uploaded through the website during submission of our manuscript. They were subsequently emailed to aes@amegroups.com and receipt was confirmed, but it appears they were not processed with our manuscript. We have uploaded the images for figures 5A and 5B and will resubmit them with this revision.

Changes in text: None; Figures 5A and 5B to be submitted with this revision.

Comment 2: Second, more description should be added to the legend for panel C (OCT image), i.e. there appear to be lucent areas in both the choroid and in the subretinal space between the retina and RPE: assuming these areas are where the lymphoma cells are located, that should be pointed out with arrows. Also, there appear to be some reflective strands in the space between the retina and RPE: what accounts for these?

Reply 2: Arrows have been added to Figure 5C to highlight the malignant cells along with further explanation in the figure legend.

Changes in text: Line 580-584: “Figure 5A-5C: Color fundus (A), autofluorescence (B) and optical coherence tomography (OCT) images (C) of a primary choroidal lymphoma. A thickened choroid can be seen on OCT with undulations and sheets of malignant cells under the retina (arrows). A large pigment epithelial detachment (asterisk) is present, which initially led to a misdiagnosis of age-related macular degeneration.”

Comment 3: Third, more description should be added to the legend for panel D, e.g., pointing out that there is lesion-corneal touch, with corneal edema. Was there secondary glaucoma?

Reply 3: Figure legend has been updated with further information.

Changes in text: Line 585-586 “Post-biopsy slit lamp image of a choroidal iris and ciliary body lymphoma with evidence of lesion-corneal touch and corneal edema.”

Comment 4: Also, was this photo taken post-biopsy (thereby accounting for the corneal sutures)?

Reply 4: Yes, legend has been updated appropriately.

Changes in text: Line 585-586 “Post-biopsy slit lamp image of a choroidal iris and ciliary body lymphoma with evidence of lesion-corneal touch and corneal edema.”

Comment 5: Line 78: Change “diffuse large B-cell lymphocytes” to “large B-lymphocytes”, first because “cell and “-cyte” are redundant, and also because the “diffuse” aspect cannot be diagnosed on a vitreous cytologic smear (which is mainly how most of these cases are diagnosed); rather, the “diffuse” label refers to the histologic pattern, which is applicable only in a solid tissue biopsy.

Reply 5: Agreed, updated appropriately.

Changes in text: Line 106 “diffuse large B-cell lymphocytes” has been updated to “large B-lymphocytes.”

Comment 6: Line 72: Mention (with added reference) that although the vast majority of cases of vitreoretinal lymphoma fall under the category of PCNSL, about 10% of cases represent dissemination of systemic lymphoma from a non-CNS site. However, of those 10%, many (particularly those of the diffuse large B-cell subtype) eventually also develop CNS disease.

Reply 6: We could not identify a source which quantified 10% or a specific percentage of vitreoretinal lymphoma cases represented by disseminated systemic lymphoma. The existing sentence in the text (line 89) emphasizes the strong correlation of PVRL with CNS disease: “More than half of PVRL patients (some estimate up to 92%) either have concurrent PCNSL at diagnosis or will eventually develop it, which makes monitoring for CNS disease critical.” The following changes were added to clarify the distinction between these diseases.

Changes in text: Line 92-94 “A minority of vitreoretinal lymphomas are not primary, i.e., they are the result of disseminated systemic lymphoma. Many of these cases however (particularly the diffuse large B-cell subtype) involve the uvea and eventually also develop CNS disease.”

Comment 7: Line 78: Mention (with added reference) that about 5% of vitreoretinal lymphoma cases are of the T- or NK/T-cell subtype.

Reply 7: We could not identify a source which quantified a specific percentage of T- or NK/T-cell subtype of vitreoretinal lymphomas. Literature demonstrates that primary vitreoretinal T-cell lymphoma is exceedingly rare, and most intraocular T-cell lymphomas result from metastatic spread from systemic lymphoma to the uvea. Line 79 – 81 addresses this issue: Cases of vitreoretinal lymphoma derived from T-lymphocytes are usually associated with metastatic spread from systemic adult T-cell

leukemia/lymphoma and are therefore secondary IOL, but very rarely PVRL of T-cell origin has been reported. An additional sentence was added prior to this to emphasize the rarity.

Changes in text: Line 106-107 “A very small minority of vitreoretinal lymphomas originate from T- or NK/T-cells.”

Comment 8: Line 80: Mention that some cases of T-cell lymphoma metastatic from a non-CNS site later also develop CNS disease.

Reply 8: Agreed, updated appropriately.

Changes in text: Line 110-111 “Some cases of T-cell lymphoma which are metastatic from a non-CNS site will also develop CNS disease.”

Comment 9: Line 84: Qualify “responds to steroid treatment”: typically the response is limited, which is one attribute that raises suspicion for intraocular lymphoma.

Reply 9: Agreed. The following changes have been made.

Changes in text: Removed portion of sentence in line 114 stating “and even at times responds to steroid treatment.” Added sentence line 114-115: “Steroids are often prescribed and typically result in no improvement, though occasionally symptoms such as floaters may temporarily improve.”

Comment 10: Line 104: Is this line supposed to contain a call-out to Fig. 4? There does not seem to be any call-out to Fig. 4 anywhere in the main body of the manuscript.

Reply 10: Yes, this has been updated.

Changes in text: “(Figure 4)” added to end of sentence at line 139.

Comment 11: Line 109: An additional, though less common, clinical finding that may be mentioned:

serous retinal detachment.

Reply 11: Agreed, updated appropriately.

Changes in text: Line 145 added “and/or serous retinal detachment.”

Comment 12: Line 140: Mention intravitreal microRNA levels as another potential diagnostic modality that has been recently reported: Upregulation of certain microRNAs was first demonstrated in the cerebrospinal fluid of CNS lymphoma patients, and subsequently, in the vitreous fluid of patients with vitreoretinal lymphoma.

Reply 12: Agreed, further information regarding microRNA levels as a diagnostic tool has been included.

Changes in text: New paragraph included at line 185-199.

Comment 13: Line 215: Mention that somewhat improved survival rates have been reported in recent years, though given the rarity of this disease, mortality rates vary widely in the literature.

A recent retrospective review reported a 5-year survival rate of 41.4%, and median survival of 38 months:

Ahmed AH, Foster CS, and Shields CL. Association of disease location and treatment with survival in diffuse large B-cell lymphoma of the eye and ocular adnexal region. JAMA Ophthalmol. 2017;135(10):1062-1068.

This somewhat higher survival rate compared to prior reports may be attributable to the most recent chemotherapy regimens; however, in the above-referenced report, it is noted that the details of the chemotherapy regimens were “not available.”

Reply 13: Agreed, updated appropriately.

Changes in text: Line 274-280 “5-year survival rates have been estimated to be less than 25% and overall survival from diagnosis has been estimated to be around 31 months or less, but in recent years the outlook for PVRL treatment may be improving [6, 24]. One recent study estimated 5-year survival at 41.4% for patients diagnosed with primary B-cell lymphoma of the eye and ocular adnexal regions.[14] The somewhat higher survival rate may be attributable to evolving chemotherapy regimens over time, but further research

will be necessary to determine this."

Comment 14: Line 246: Define the abbreviation "IGA" by putting it in parentheses immediately after the spelled-out version.

Reply 14: Agreed, updated appropriately.

Changes in text: Line 327 "indocyanine green angiography (IGA)[46]"

Comment 15: Line 257: Add ", and" in between "PCR" and "immunohistochemistry."

Reply 15: Agreed, updated appropriately.

Changes in text: Line 348 "Flow cytometry, PCR and immunohistochemical staining for B-cell markers are useful to confirm cellular lineage and monoclonality."

Comment 16: Line 265: Fix the comma splice before "however": the comma should be replaced with a semi-colon or period.

Reply 16: Agreed, updated appropriately.

Changes in text: Line 355-356 "Uniform CT or MRI enhancement is typical in cases which have extrasceral extension. However, these imaging modalities are less useful due to the ill-defined appearance."

Comment 17: Line 288: Change "from choroidal circulation" to "through choroidal circulation" (because ultimately, the lymphoma dissemination is coming "from" the vasculature of the primary site).

Reply 17: Agreed, updated appropriately.

Changes in text: Line 398 "The choroid is the most common site to be affected by secondary IOL, likely through hematogenous spread through choroidal circulation."

Comment 18: Fig. 1A legend: Replace “retinal” with “retinal/subretinal.”

Reply 18: Agreed, updated appropriately.

Changes in text: Line 568 “Color fundus picture of vitreoretinal lymphoma with a large placoid retinal/subretinal mass.”

Comment 19: Fig. 2 legend: Replace “hypopyon” with “pseudohypopyon.”

Reply 19: Agreed, updated appropriately.

Changes in text: Line 572 “Natural killer T-cell intraocular lymphoma with a blood-tinged, tan pseudohypopyon.”

Comment 20: Fig. 4c legend: Add more clarity to the description: By “drusenoid changes,” do the authors mean to say that there are sub-RPE clumps of lymphoma cells simulating drusen? Also, by “loss of inner retinal architecture,” do the authors mean to say that the lymphoma cells are invading the inner retina? Also, please point out these findings with arrows.

Reply 20: Arrows have been added to the image along with further explanation of the findings.

Changes in text: Line 577-579 “Macular OCT showing drusenoid changes under the temporal macula (arrows) and loss of inner retinal architecture consistent with retinal infiltration (asterisk) in a patient with vitreoretinal lymphoma.”

Reviewer B

Comments:

Nice review regarding an important topic, which has to be improved before publication. Please follow up my recommondations precisely to make sure, that this manuscript is more likely suitable for this journal.

Comment 1:

Abstract:

Include the difference between uveal melanoma and vitreoretinal lymphoma here.

Reply 1: It is presumed that ‘uveal melanoma’ was intended to be ‘uveal lymphoma.’ The following sentences have been included in the abstract to further delineate the difference between vitreoretinal and uveal lymphoma.

Changes in text: Line 39-45 “Primary vitreoretinal lymphomas generally originate from B-lymphocytes and are associated with central nervous system lymphoma. Ophthalmic findings include retinal pigment epithelium changes with yellow subretinal deposits known as “leopard spotting.” Primary uveal lymphomas generally originate from low-grade B-lymphocytes invading the choroid and carry an improved prognosis compared to vitreoretinal lymphomas. Funduscopic findings of primary uveal lymphoma include yellow to pink-yellow choroidal swelling with infiltrative subconjunctival “salmon-patch” lesions."

Comment 2:

2. Introduction

Line 52 Include citation PMID: 32585164

Name all potential affected uveal structures (Iris, ciliary body, choroid) here.

Define the expression intraocular lymphoma with its subgroups in detail.

Point out the difficulties to discriminate by clinical signs from other disease and also mention, that lymphoma is often late diagnosed for these reasons.

The objective might be removed for this narrative review.

Reply 2:

Citation has been included. Uveal structures have been named. Further details and explanation of IOL subgroups have been added. The diagnostic difficulty and subsequent delay in diagnosis is discussed. Objective has been removed.

Changes in text:

Citation PMID 32585164 included at line 63.

Line 64-65 “Uveal lymphoma is that which affects the iris, ciliary body and/or choroid while vitreoretinal

lymphoma affects the retina and/or vitreous."

Line 66-75 “Primary vitreoretinal lymphoma (PVRL) is associated with development of central nervous system lymphoma before, after or concurrent with diagnosis. It should be suspected in immunosuppressed patients of advanced age presenting with vision changes and floaters which do not respond to steroids. Uveal lymphoma is unrelated to CNS lymphoma yet arises in patients of advanced age with blurred vision or metamorphopsia with a predilection for males. The clinical presentation of all types of intraocular lymphoma may be difficult to distinguish from uveitis, degenerative changes, and other ocular diseases and for these reasons diagnosis is often delayed. We review the current literature regarding intraocular lymphomas and their management to increase disease recognition of this important entity.”

Objective removed after line 75.

Comment 3:

3. Methods

The search should be expanded with iris lymphoma, choroidal lymphoma and ciliary body lymphoma.

Reply 3: Agreed, updated appropriately.

Changes in text:

Line 77-79 “Online databases PubMed and Embase were searched using the terms ‘intraocular lymphoma’, ‘vitreoretinal lymphoma’, ‘uveal lymphoma’, ‘iris lymphoma’, ‘choroidal lymphoma’, and ‘ciliary body lymphoma’ published from 1990 to June 2021.”

Comment 4:

4. Summary/Discussion

Line 74/75 remofe after the age of 40. There are also known cases before 40.

Line 85 add pirmary before CNS lymphoma

LIne 123 correct to 30% according to the International Primary CNS Lymphoma Collaboration Group PMID: 22045784

Line 140 Include an paragraph with miRNA as a possible tool for diagnostics in PVRL(e.g. PMID: 24345320, PMID: 32545709, PMID: 28389463)

Please explain in detail, when to go for chorioretinal biopsy.

Line 201 Mention the intraocular side effects of MTX here (corneal toxicity, retinal toxicity). Include here the use of intravitreal Rituximab with potential less side effects (e.g PMID: 24049708, PMID: 17464308, PMID: 33945240, PMID: 26450638).

I am missing a work up and follow up for PVRL and for uveal lymphoma.

There is almost no information on iris and ciliary body lymphoma (diagnosis, treatment, work up, follow up). This has to be added.

Also include a outlook for the future at the end.

Reply 4:

Line 97 reference to PVRL arising in patients above the age of 40 has been removed.

Primary has been added before CNS lymphoma at line 116.

Line 161 statistic updated to 30% with PMID 22045784 citation included.

Paragraph discussing microRNA as a possible diagnostic tool for PVRL has been included with relevant citations, line 185-199.

Line 208-211 further information regarding indications for chorioretinal biopsy has been included.

Sentences discussing toxicity and adverse effects of intravitreal methotrexate therapy and rituximab as an alternative have been moved from line 271 to line 258 with additional information added.

Follow up guidance for PVRL is included at line 281-283. Follow up guidance for uveal lymphoma is included at line 368-382.

Characteristics, diagnosis, work up and follow up for primary iris lymphoma has been included within the section titled Primary Uveal Lymphoma, lines 285-382. Primary ciliary body lymphoma is discussed line

288-290; given the lack of data we do not comment on it as an individual entity.

Outlook for the future is included at line 435-436.

Changes in text:

Line 96-97 “PVRL appears to affect both genders equally and commonly arises in the 6th decade of life.”

Line 115-117 “It is important to remember that primary central nervous system lymphoma may be diagnosed prior to PVRL, be detected simultaneously, or develop after diagnosis.”

Line 160-163 “Due to these challenges, false negatives are relatively common (up to 30%) and patients may require multiple biopsies or undergo chorioretinal biopsy in order to identify malignant lymphocytes for definitive diagnosis.”

Paragraph discussing microRNA as a diagnostic tool for PVRL is included at lines 185-199.

Line 208-211 “It may be indicated for patients with high remaining suspicion of PVRL despite inconclusive diagnostic vitrectomy results, patients with disease unresponsive to treatment, and patients whose disease may not involve the vitreous.”

Line 258-265 “Rituximab is increasingly utilized as chemotherapy, both systemically and intravitreally, as research suggests it may offer less toxicity in comparison to methotrexate. Adverse effects of intravitreal rituximab include transient intraocular pressure elevation and iridocyclitis. Intravitreal methotrexate has been associated with significant retinal and corneal toxicity. Keratopathy is a frequent adverse effect of intravitreal methotrexate which may become severe, manifesting as blurred vision, redness, photophobia and excessive tearing.”

Line 287-305 “Primary lymphoma of the ciliary body alone is almost unmentioned in current literature and will be discussed under the broader context of primary uveal lymphoma in this paper. Primary lymphomas affecting only the iris exist in a handful of case reports, after excluding the more frequent cases of iris

lymphoma which are suspected to have arisen secondary to systemic or choroidal lymphoma. Cases of suspected primary iris lymphoma are often high-grade B-lymphocytic neoplasms which carry poor prognosis."

Line 312-315 "Cases of primary choroidal lymphoma appear to show a predilection for males, ages 50-70, who present with blurred vision or metamorphopsia. Occasionally patients may be asymptomatic, and disease is detected on ophthalmologic exam. Primary iris lymphoma is often misdiagnosed as anterior uveitis, given the overlapping symptoms of blurred vision, redness and eye pain."

Line 323-325 "Features indicative of iris lymphoma rather than anterior uveitis include hyphema, abnormal iris vessels, and the aforementioned salmon-patch conjunctival lesions."

Line 361-363 "Anterior segment optical coherence tomography (OCT) and ultrasound biomicroscopy are useful tools to differentiate lymphoma of the iris from anterior uveitis, though biopsy is ultimately necessary for definitive diagnosis."

Line 368-377 "Management of primary uveal lymphoma is nonstandardized given its rarity and most often involves discussion via multidisciplinary tumor board. Treatment with external beam radiotherapy (EBRT) may induce complete, lasting remission for primary choroidal lymphoma and is well tolerated. Prognosis is generally excellent for primary choroidal lymphomas which are histologically EMZL, with small studies finding 5-year survival rates to be 100% and rates of complete remission up to 79% in cases managed with either radiotherapy or rituximab. Given the lack of literature specific to primary iris and/or ciliary body lymphoma and the lack of certainty that existing cases are truly primary lymphomas, it is difficult to recommend optimal management or predict prognosis."

Line 435-436 "Future outlook for treatment and prognosis of intraocular lymphoma is likely to improve with less invasive molecular diagnostic techniques and increased awareness."

Comment 5: Fig 3 Name the disease.

Reply 5: Agreed, updated appropriately.

Changes in text: Line 574 “Yellow subretinal deposits with overlying retinal pigment epithelium “leopard spotting” seen in primary vitreoretinal lymphoma.”

Comment 6: Fig 4 Use arrows to mark the lesions.

Reply 6: Agreed, updated appropriately.

Changes in text: Arrows have been added to figure 4A and 4C.

Comment 7: Fig 5 is not shown in the PDF.

Reply 7: Figure 5A and 5B were unfortunately unable to be uploaded through the website during submission of our manuscript. They were subsequently emailed to aes@amegroups.com and receipt was confirmed, but it appears they were not processed with our manuscript. We have uploaded the images for figures 5A and 5B and will resubmit them with this revision.

Changes in text: None; figures 5A and 5B to be resubmitted with this revision.

Reviewer C

Comments:

Due to the recent editorial update on the regulations of manuscripts (https://aes.amegroups.com/pages/view/guidelines-for-authors#content-2-2-2), would you please further revise the manuscript? Below please see the detailed comments.

1.Abstract

Structured, 4 parts are Background and Objective, Methods, Key Content and Findings, Conclusion

(1) Please combine "Objective" and "Background" into a subsection entitled "Background and Objective".

(2) Before Conclusions, please add Key Content and Findings to describe what the literature review will mainly contain and any key findings during the literature review.

1. Reply: This has been updated as requested.

1. Changes in text:

Line 24: Background and Objective

Line 31: Methods

Line 35: Key Content and Findings

Line 57: Conclusion

2. Methods

It's great that the authors report the methods of search. In addition to reporting the databases, timeframe, search terms and Inclusion and exclusion criteria (line 76-81), please also present the date of search, detailed search strategy and selection process.

To further make the information more easy-going and self-explaining, please include a completed table (https://aes.amegroups.com/pages/view/guidelines-for-authors#content-2-2-2) in the Methods section. This part is essential as it reflects the sources of evidence (even though it is not a systematic review). This is to transparently report the process, not to judge it.

2. Reply: The Methods section has been updated with additional requested information and the requested table.

2. Changes in text: Methods section, line 89 – 94. See Table 1, page 25.

3. Discussion

We strongly suggest the authors draw a table to summarize the key findings of IOL (e.g. ophthalmic findings, diagnosis and management of three types of IOL). This would clearly save readers' time and highlight the key points.

3. Reply: We agree; we have created Table 2 and hope this supplies readers with an easily accessible summary of the article's information.

3. Changes in text: Table 2, page 25.

4. Abbreviations

Please provide the full name of the abbreviations that first appears in this article (e.g. "PCR" in line 166, "CSF" in line 203). Please check through the manuscript to address similar concerns.

4. Reply: We have reviewed the manuscript and added full explanations of each abbreviation used.

4. Changes in text: PCR in line 179-180, MRI in line 214-215, CSF in line 216, IGA in line 352, CT in line 372, PET in line 381-382, DLBCL in line 421; included key of various abbreviations used in Table 2.