Primary vitreoretinal lymphoma: A diagnostic and management challenge.

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Abstract:
Primary vitreoretinal lymphoma (PVRL) is a rare form of primary central nervous system lymphoma (PCNSL) arising in the intracocular compartment without brain involvement. Despite its apparent indolent clinical course, PVRL can cause permanent vision loss and CNS relapse, the major cause of death in PVRL patients. The pathophysiology of PVRL is unknown. As in PCNSL, the transformation of the tumor cells likely originates outside the CNS, before the cells migrate to the eye and proliferate within an immune-permissive microenvironment. PVRL exhibits a biased immunoglobulin repertoire, suggesting underlying antigen selection. The diagnosis remains challenging, requiring close coordination between ophthalmologists and cytologists. Because of their rarity and fragility in the vitreous, lymphoma cells cannot always be identified. Interleukin levels, molecular biology and imaging are used in combination with clinical ophthalmological examination to support the diagnosis of PVRL.

Multi-institutional prospective studies are urgently needed to validate the equivocal conclusions regarding treatments drawn from heterogeneous retrospective or small cohort studies. Intravitreal injections of methotrexate or rituximab or local radiotherapy are effective at clearing tumor cells within the eyes but do not prevent CNS relapse. Systemic treatment based on high-dose methotrexate chemotherapy, with or without local treatment, might reduce this risk. At relapse, intensive consolidation chemotherapy followed by stem cell transplantation can be considered. Single-agent ibrutinib, lenalidomide and temozolomide treatments are effective in patients with relapsed PVRL and should be tested as first-line treatments. Therapeutic response assessment based on a clinical examination is improved by measuring cytokine levels but still needs to be refined.

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Abstract

Primary vitreoretinal lymphoma (PVRL) is a rare form of primary central nervous system lymphoma (PCNSL) arising in the intraocular compartment without brain involvement. Despite its apparent indolent clinical course, PVRL can cause permanent vision loss and CNS relapse, the major cause of death in PVRL patients. The pathophysiology of PVRL is unknown. As in PCNSL, the transformation of the tumor cells likely originates outside the CNS, before the cells migrate to the eye and proliferate within an immune-permissive microenvironment. PVRL exhibits a biased immunoglobulin repertoire, suggesting underlying antigen selection. The diagnosis remains challenging, requiring close coordination between ophthalmologists and cytologists. Because of their rarity and fragility in the vitreous, lymphoma cells cannot always be identified. Interleukin levels, molecular biology and imaging are used in combination with clinical ophthalmological examination to support the diagnosis of PVRL.

Multi-institutional prospective studies are urgently needed to validate the equivocal conclusions regarding treatments drawn from heterogeneous retrospective or small cohort studies. Intravitreal injections of methotrexate or rituximab or local radiotherapy are effective at clearing tumor cells within the eyes but do not prevent CNS relapse. Systemic treatment based on high-dose methotrexate chemotherapy, with or without local treatment, might reduce this risk. At relapse, intensive consolidation chemotherapy followed by stem cell transplantation can be considered. Single-agent ibrutinib, lenalidomide and temozolomide treatments are effective in patients with relapsed PVRL and should be tested as first-line treatments. Therapeutic response assessment based on a clinical examination is improved by measuring cytokine levels but still needs to be refined.
INTRODUCTION

Primary vitreoretinal lymphomas (PVRLs) arise in the intraocular compartment without brain involvement, thus constituting a rare subgroup of primary central nervous system lymphoma (PCNSL). Due to the clinical similarities to uveitis and initial response to steroid therapy, the PVRL diagnosis may be delayed. The initial indolent clinical course must not minimize the severity of this disease, which can result in permanent vision loss or CNS relapse with a poor prognosis. Therapeutic options range from local ocular treatment to systemic therapy based on high-dose methotrexate (HD-MTX) and even intensive chemotherapy with autologous stem cell transplantation (IC+ASCT). The diagnostic procedure is improved by analyses of biomarkers and molecular biological parameters. Efforts are needed to better assess the therapeutic response.

This review aims to summarize the most important studies in the field of PVRL, including their pitfalls and limitations, to assist with decision-making in clinical practice and to better identify the next challenges to address in this disease.

DEFINITION AND EPIDEMIOLOGY

PVRL is a rare high-grade extranodal non-Hodgkin lymphoma affecting the vitreous, retina or, exceptionally, the optic nerve in the absence of brain parenchyma infiltration.

PVRL must be distinguished from secondary vitreoretinal or choroidal invasion by a systemic lymphoma and from primary uveal lymphomas, which arise in the choroid, iris or ciliary body and are mainly MALT (mucosa-associated lymphoid tissue) lymphomas (figure 1). The vitreoretinal involvement present at the diagnosis of a PCNSL, which occurs in 15% of patients with PCNSL, falls outside the definition of PVRL.

The majority of PVRLs are high-grade diffuse large B-cell lymphomas (DLBCLs). Analogous to PCNSL, one can suggest that PVRL also belongs to the activated-B cell type (ABC), more specifically to the subgroup of ABC-DLBCL, with frequent mutations in CD79 and MYD88L265P and a less favorable outcome than other ABC subgroups. Exceptional unclassifiable B-cell lymphoma, follicular lymphoma and T-cell lymphoma are encountered.

True epidemiological data are lacking. The PVRL incidence is estimated to be 50 cases/year in the US. PVRL represents approximately 5% of the patients registered in the French
database for oculo-cerebral lymphomas and accounts for 10 new cases/year\(^3\),\(^8\). PVRL usually occurs in immunocompetent adults during the fifth decade with a slight female predominance but no racial predilection\(^4\),\(^9\). Some cases have been described in patients with immunosuppression, usually associated with Epstein-Barr virus\(^10\). PVRL is mainly bilateral although asymmetric presentations are encountered.

**PATHOPHYSIOLOGY OF PVRL AND CNS RELAPSE**

PVRL pathophysiology remains to be deciphered. The lymphoma cell origin is unknown, but similarly to PCNSL, the transformation of the tumor cells likely originates outside the CNS, before the cells migrate to the eye and proliferate. The microenvironments of the CNS and the eyes share similar characteristics. The retina is isolated from the vascular and perivascular compartment by two blood–retinal barriers (BRBs), composed of cells linked by tight junctions at the level of the intraretinal capillary endothelial cells (inner BRB) and the retinal pigmented epithelium (RPE) (outer BRB), which substantially restrict paracellular diffusion of molecules into the retina from the vascular and perivascular compartments, respectively\(^11\). The ocular microenvironment shows low expression of major histocompatibility complex class molecules and is enriched in immunosuppressive molecules, such as transforming growth factor-beta, macrophage migration inhibitory factor and Fas ligands, which suppress the activation of Th1 cells and the inflammatory activity of macrophages and prevent natural killer cell activation. The secretion of interleukin-10 (IL-10), an immunosuppressive cytokine, by lymphoma cells may increase immune tolerance and allow tumor cells to escape the immune system\(^12\),\(^13\). Data on ocular immune reactions to tumor cells are scarce. Impaired Th1 cytokine production by tumor-infiltrating lymphocytes has been documented in PVRL animal models\(^13\).

According to a recent study\(^14\), PVRLs displayed very high levels of immunoglobulin (IG) gene mutations, especially in the IGHV4-34 sequences, and in a significantly higher proportion than in PCNSL. This biased IG repertoire suggests that antigen selection plays a major role in PVRL development and highlights a potential role for inhibitors of the B-cell receptor (BCR) signaling pathway in blocking the activity of the NF-κB pathway, which is activated in ABC-DLBCL\(^15\). The galectin-3 protein, which is recognized by antibodies using the IGHV4-34 gene
and is expressed in cells from both the brain microenvironment and RPE, is a candidate antigen\textsuperscript{14,16}. Further studies are needed to identify the antigen responsible for PVRL.

The pathophysiology of CNS relapse from PVRL is poorly documented. Whether CNS relapse results from subclinical brain disease involvement that is not detectable with routine magnetic resonance imaging (MRI) at diagnosis or from dissemination from the eye through the retina and optic nerve is still a matter of debate. Experiments using murine models\textsuperscript{17,18} support the hypothesis of dissemination through the RPE into the choroid, thus highlighting the importance of achieving complete remission in the eye. The detection of IL-10 in the cerebro-spinal fluid (CSF) of patients with PVRL, without CSF or brain involvement of lymphoma\textsuperscript{19}, might suggest a subclinical brain involvement of the disease\textsuperscript{19}.

**DIAGNOSIS**

Distinguishing PVRL from posterior uveitis is still a challenge for ophthalmologists. PVRL is part of the well-named masquerade syndrome: an insidious onset, initial response to steroids and delayed diagnosis due in part to the common prolonged and indiscriminate use of steroids are common\textsuperscript{4}. The median time from first symptoms to diagnosis ranges from 6 to 40 months\textsuperscript{9,20,21} compared to 35 days for PCNSL\textsuperscript{22}. Patients may complain of floaters or blurred vision\textsuperscript{4}. A slit-lamp examination reveals a quiet anterior chamber with no protein flare and some cellular gray diffuse keratic precipitates. Lymphoma cell clumps are noticeable in the anterior vitreous or along the vitreous fibrils. Lymphoma cells may also infiltrate the retina (multifocal cream-colored retinal spots on funduscopic imaging) or grow along the Bruch membrane under the RPE. Multimodal retinal imaging reveals the typical but nonpathognomonic leopard skin pattern, while subretinal cell deposits are highlighted with noninvasive optical coherence tomography (OCT) (figure 2). Clinical findings and paraclinical signs of PVRL and uveitis are summarized in table 1.

Although an ophthalmic examination and ocular imaging might raise clinical suspicion\textsuperscript{23}, the gold standard for PVRL diagnosis is still the cytological identification of lymphomatous cells in the eye (figure 3). A diagnostic vitrectomy is usually performed. Less frequently, a retinal biopsy is necessary. A cytological examination, immunocytochemistry, flow cytometry, measurement of cytokine levels and molecular examinations of these samples should be combined to improve the sensitivity and specificity of the PVRL diagnosis\textsuperscript{10}. The diagnosis
could remain challenging owing to a limited sample volume, low cellularity of vitreous fluid samples and the extreme fragility of lymphoma cells in the vitreous. The procedures for vitreous sampling and analysis have been described previously 24–27 (table 2). We must emphasize that for a successful diagnostic procedure the discontinuation of steroids several weeks before vitrectomy, good communication and collaboration between the ophthalmologist and the cytologist/pathologist trained in handling vitreous samples, and an immediate analysis of both pure and diluted vitreous samples preferentially without a fixative are critical.

Cytology provides morphological evidence of PVRL. Owing to the difficulties of sampling, the sensitivity of cytology alone in diagnosing PVRL is low (45–60%) 28. Immunohistochemistry or flow cytometry of pan B-cell markers can show a CD20+ population of B-cells with monotypic light chain expression 29,30. Monoclonality can be identified by polymerase chain reaction (PCR) to detect immunoglobulin heavy-chain or Kappa light-chain gene rearrangement 31–33. Results of flow cytometry and PCR analyses must be interpreted with caution in samples with low cellularity.

IL-10 measurements in pure vitreous or aqueous samples are a significant diagnostic aid when PVRL is clinically suspected. High IL-10 levels in vitreous or anterior chamber favor a diagnosis of PVRL, while high IL-6 levels are more specific to inflammatory or infectious diseases. The IL-10/IL-6 ratio is thus very helpful in determining the PVRL diagnosis 34–36 while avoiding errors related to sample dilution. Caution remains for pure retinal localization, which may not be accompanied by an increase in IL-10 in intraocular fluids. Cassoux et al. 12 defined a specific cutoffs of 50 pg/mL IL-10 in the aqueous humor (sensitivity of 89%; specificity of 93%) and 400 pg/mL in the vitreous (sensitivity of 80%; specificity of 99%) (figure 4). More recently, an Interleukin Score for intraOcular Lymphoma Diagnosis (ISOLD) was proposed as a probability score for the diagnosis of PVRL (figure 4) 37, which combines the IL-10 and IL-6 levels. Assessments of interleukin levels are currently routine procedures for the screening and follow-up of PVRL in many referral centers, as an anterior chamber tap is a rapid and minimally invasive procedure 38.

*MYD88* and *CD79B* gene mutations are frequently reported in PCNSL 7. The most common mutation, *MYD88* 1265P, increases NF-κB activity, which promotes cell survival 39. The *MYD88* 1265P mutation was observed in the vitreous in up to 88% of a series of 25 patients with PVRL 40. Adding the *MYD88* gene mutation test might improve the diagnostic yield of
Concomitant *MYD88* mutations were detected in paired aqueous and vitreous samples from small series of patients. *CD79B*, which encodes the BCR, was mutated in 35% of a series of 17 patients with PVRL. Yonese et al. suggested an association between the *CD79B* mutation and a poorer prognosis due to earlier CNS involvement. Recent epigenetic studies identified microRNAs such as miR-6513-3p and miR-1236-3p in the vitreous and serum that might serve as candidate PVRL biomarkers. Whole-exome sequencing to determine the PVRL mutation profile has recently been conducted but small targeted gene panel tests remain to be developed.

In summary, a definite PVRL diagnosis relies on the cytological identification of malignant lymphoid cells with monotypic and/or clonal characteristics. This diagnosis may be challenging when cytological evidence of PVRL is lacking because of a low-cellularity or poor-quality sample. In these cases, the PVRL diagnosis will be highly probable based on evidence from multiple tests combining an ophthalmological clinical examination, imaging (such as angiography or OCT) and measurements of IL-10/IL-6 levels for ISOLD scoring.

Cerebral MRI, CSF examination including cytology and ideally flow-cytometry for lymphoid markers, CT or PET/CT of chest/abdomen and pelvis, and bone marrow biopsy, are necessary in the initial disease assessment to rule out brain and extraCNS lymphoma involvement.

**THERAPEUTIC ISSUES**

Therapeutic challenges for PVRL are dual. Local and CNS relapses are frequent. Fifty-six to 90% of patients with PVRL ultimately develop CNS dissemination within 30 months. Median progression-free survival (PFS) ranges from 18 to 29 months. Overall survival (OS), ranging from 58 to 75 months, is however longer than the estimated survival of patients with PCNSL. Therapy aims to treat the intraocular and retinal disease to restore the patient’s vision and to decrease the incidence of CNS relapses, which represent the main cause of death.

Different treatment strategies are available: local treatment, such as intravitreal (IVT) injection of chemotherapy or ocular radiotherapy (ORT); systemic treatment; or a
combination of both. Debate persists regarding which strategy to adopt as the first-line treatment. The large number of published studies reflects the efforts of medical teams to improve the prognosis of patients with PVRL. However, many pitfalls hinder their interpretation. The largest retrospective studies involved patients who received multiple treatments, resulting in small subgroups receiving similar treatments. The few prospective studies available were conducted in a very limited number of patients. Interestingly, the median survival appears longer in patients treated at the time of the PVRL diagnosis than in patients treated at the time of CNS relapse.

How patients are monitored for the early detection of an ocular or CNS relapse is usually not specified in the published series. Differences in monitoring procedures may account for part of the observed differences in PFS.

**First-line treatments**

**Local treatments**

The goal of local treatment is to induce intraocular complete remission (CR) and improve vision without systemic toxicity. Studies have not proved whether the achievement of a local CR reduces the risk of CNS relapse.

The IVT route for chemotherapy is an interesting procedure to rapidly reach intraocular cytotoxic drug concentration. Data on the ocular bioavailability of IVT chemotherapy are scarce. De Smet et al. showed that a single IVT injection of 400 µg of methotrexate remained effective for more than 5 days (table 2). Kim et al. determined that the half-life of rituximab in the rabbit vitreous (IVT injection of 1 mg/0.1 mL) is 4.7 days. IVT injections are a safe procedure performed under topical anesthesia. Methotrexate is the main drug used for IVT injection at a dose of 0.4 mg/0.1 mL. Various IVT administration schedules have been published (supplemental data). The induction phase consists of twice-weekly IVT for 4 weeks. The treatment is then followed by a predetermined number of IVT injections (for a total of 25 IVT injections over one year) or driven by the clinical response or the IL-10 level in the aqueous humor to decrease the number of IVT injections. IVT rituximab injections at a dose of 1 mg/0.1 mL, alone or combined with IVT injections of methotrexate, are an interesting alternative that would require fewer injections to control PVRL. Protocols vary between authors. Some authors also reported IVT injections of melphalan.
The main IVT methotrexate-related side effects are transient intraocular hypertonia, epithelial keratopathy\textsuperscript{63,64} and, in some cases, drug resistance\textsuperscript{71}. Reversible uveitis may occur after IVT rituximab\textsuperscript{68}. These side effects are reduced by performing an anterior chamber tap before the IVT injection to reduce intraocular pressure and drug reflux and abundant washing of the cornea with saline. The obtained aqueous sample is then used to monitor the IL-10 level. Intraocular hemorrhage, retinal detachment or endophthalmitis are rare vision-threatening side effects caused by the procedure of IVT injections (0.06, 0.01 and 0.05% of injections, respectively)\textsuperscript{72}.

IVT chemotherapy has been used as first-line treatment, while some authors use IVT chemotherapy only for ocular relapses or when systemic chemotherapy is contraindicated\textsuperscript{4,66,73}. The efficacy of IVT chemotherapy as the sole first-line treatment for PVRL cannot be properly assessed because of the heterogeneity encountered in retrospective studies in terms of disease characteristics (initial PVRL or relapse, vitreo-retinal involvement associated with PCNSL) and the frequent association of systemic treatments.

External beam ORT (30-40 Gy) is an alternative local treatment although it is rarely offered as the only first-line treatment\textsuperscript{4}. It is effective for local control of the disease and may be preferable in patients with bilateral disease\textsuperscript{4}, but fails to prevent the CNS relapse. The risk of cataract and radiation retinopathy decreases with lower doses (< 30 Gy) of ORT\textsuperscript{9,58,66,74–76}.

Therapeutic vitrectomy can be considered a palliative treatment to improve vision\textsuperscript{77}.

**Systemic and combined (local + systemic) treatments**

Systemic treatments are empirical and mainly rely on PCNSL-like treatments with HD-MTX, based on the anatomical and functional similarities between the blood–brain barrier and the BRB\textsuperscript{11}. Knowledge of the pharmacokinetic of drugs administered systemically in ocular tissues and fluid is scarce; it has been explored in a few studies of a small number of patients (table 3). After IV HD-MTX and aracytine administration, these drugs were found in the aqueous humor and to a lesser extent in the vitreous shortly after the injection\textsuperscript{78–80}. Ifosfamide and trofosfamide were detectable in the aqueous humor after IV and oral administration respectively\textsuperscript{81}, but the data in the vitreous are lacking for these drugs.
The benefit of combining a first-line treatment with systemic HD-MTX chemotherapy and local treatment to decrease the risk of CNS relapse is controversial based on the results of heterogeneous retrospective studies\(^9,50–57,82\) (table 4) that aimed to compare the outcome of patients who received only local treatment with the outcomes of patients who received systemic treatment or a combination of both. The retrospective study by Hashida\(^55\), in which the treatments were more homogeneous, showed significantly delayed CNS relapses in patients who received a combination of local and systemic HD-MTX treatment. Due to the aforementioned biases of the published therapeutic studies in patients with PVRL, the failure of retrospective studies to prove the superiority of the combined therapeutic approaches should not discourage clinicians from testing treatments with the objective of decreasing CNS relapse. Kaburaki et al.\(^57\) reported encouraging results from a small prospective series of 11 patients treated with a conventional PCNSL-like treatment consisting of systemic IV RMPV (rituximab, HD-MTX, procarbazine, vincristine) + reduced-dose whole-brain radiotherapy (23.4 Gy) + IV HD-AraC combined with IVT-MTX. With a median follow-up of 40 months, the estimated 4-year cumulative incidence of CNS progression was only 10%. The combination of bilateral ORT (30-40 Gy) with systemic HD-MTX produced encouraging survival results in a cohort of 11 patients, but resulted in radiation retinopathy in 9 patients (with optic atrophy \((n = 2)\) or cataract \((n = 2)\)) and bilateral optic nerve atrophy in one patient\(^82\).

Treatments for relapsed or refractory PVRL

At relapse, IVT chemotherapy and ORT can be provided as a single treatment with the same objectives as discussed in first-line treatment or to rapidly improve vision in combination with systemic treatment.

Intensive chemotherapy followed by autologous stem cell transplantation (IC+ASCT)

IC+ASCT has been evaluated in patients with refractory or relapsed (R/R) PVRL to circumvent the blood–retina barrier\(^83–85\), mainly with an IC regimen consisting of HD thiotepa-busulfan-cyclophosphamide, because this combination of drugs proved feasible and effective in patients with poor-prognosis systemic lymphomas, including patients with CNS disease involvement\(^86\), and because thiotepa and busulfan have good CNS bioavailability\(^87\). IC+ASCT proved feasible in patients with R/R PVRL (table 5). A pilot study reported encouraging results for patients with refractory PVRL after induction chemotherapy based on HD-MTX
and HD-AraC\textsuperscript{83}: the 5 patients who received IC+ASCT achieved a CR and no CNS relapse was observed after ASCT with a median follow-up of 17 months. Patients with R/R PVRL were included in subsequent retrospective\textsuperscript{85} and prospective\textsuperscript{84} studies for R/R PCNSL and PVRL with the same IC+ASCT regimen. These studies confirmed the efficacy of this therapeutic procedure in patients with R/R PCNSL and PVRL, with no significant different outcomes between patients with PVRL and PCNSL. Other retrospective studies\textsuperscript{52} also suggested a beneficial effect of IC+ASCT on PVRL. Although these statements are based on a small series of patients with PVRL, due to the rarity of this disease, and in the absence of any comparative studies, IC+ASCT can still be considered a treatment option for relapsed PVRL in younger patients with no severe comorbidities.

**Single agents and targeted therapies (table 5)**

Single-agent temozolomide produced encouraging results in a retrospective study involving 21 patients (relapse, n= 19; first-line, n = 2)\textsuperscript{88}. The overall response rate (ORR) was 81%, with 71% achieving a CR. The toxicity profile was good. With a median follow-up of 42 months, the median PFS was 12 months and 5 CNS relapses occurred.

Two targeted therapies, lenalidomide as a single agent or combined with rituximab, and ibrutinib, showed clinical activity in the ocular compartment in phase I and prospective “proof-of-concept” phase II studies\textsuperscript{89–92} (table 5). These therapies were tested because of their known efficacy against systemic DLBCL, with preferential antilymphoma activity against the ABC-DLBCL subtype\textsuperscript{5,14,93,94}.

In the REVRI study\textsuperscript{89}, the induction treatment consisted of eight 28-day cycles of R2 (rituximab 375/m\textsuperscript{2} IV D1; lenalidomide 20 mg/day, D1-21 for cycle 1; and 25 mg/day, D1-21 for the subsequent cycles). Fifty patients were recruited in the study, including seventeen patients with intraocular disease involvement (11 patients with R/R PVRL and 6 patients with disease involvement of the brain and eyes). In this patient subgroup, the CR rate was 35%. The median PFS of patients with PVRL was 9.2 months. The median OS of patients with PVRL was not reached and was significantly longer than that of patients with PCNSL (p=0.03). Although the precise roles of rituximab and lenalidomide in determining the therapeutic
outcomes in this population cannot be defined, this study documented the clinical activity of the combination of rituximab and lenalidomide in the ocular compartment.

The prospective multicenter phase II iLOC study\textsuperscript{90} evaluated the activity of ibrutinib as a single agent (560 mg/day every day) in patients with R/R PCNSL and PVRL. Fourteen patients with R/R PVRL were included. After 2 months of treatment, an ORR was observed in 86\% of patients with intraocular disease involvement, including CR in 50\%. The median PFS of patients with PVRL was 23 months. After a median follow-up of 26 months, one CNS relapse was observed.

**ASSESSMENT OF THE THERAPEUTIC RESPONSE AND FOLLOW-UP**

According to IPCG\textsuperscript{95}, the definition of an ocular CR requires “no evidence of active ocular lymphoma as defined by the absence of cells in the vitreous and resolution of any previously documented retinal or optic nerve infiltrates”. In practice, the response to treatment is assessed by a clinical ophthalmological evaluation\textsuperscript{96}, which aims to assess the reduction in vitreous and retinal invasion by tumor cells. However, this evaluation is subjective. The ophthalmological definitions of CR, partial response and relapse are difficult to determine since a measurable mass is not present. Tumor vitreous haze and retinal infiltration are observer-dependent measures. True CR is difficult to ascertain, as many patients present minimal residual disease, which is difficult to distinguish from an active infiltrate\textsuperscript{97}. Assessment of the PVRL response is a major challenge. Indeed, PVRL is currently the only aggressive lymphoma for which the assessment of a response to treatment is based on a clinical evaluation, which differs substantially from the recommendation for systemic lymphoma\textsuperscript{98}.

Ancillary ophthalmological examinations, such as OCT, could provide interesting noninvasive, reproducible and comparative information about the evolution of retinal lesions (figure 5), as evidenced by a retrospective case analysis\textsuperscript{96}. However, multimodal imaging including retinal photography, angiography and OCT enables an assessment of the posterior pole of the eye and the retinal lesion, but not the vitreous infiltration.

IL-10 is a promising biomarker. Exploratory studies have shown that the decrease in IL-10 levels in the aqueous humor is a minimally invasive indicator of the treatment response, while an increase might suggest recurrence\textsuperscript{66}. This biomarker might facilitate early relapse
detection. Prospective studies evaluating specific correlations between IL-10 levels and clinical CR, PFS or OS are lacking.

After CR is achieved, rigorous follow-up is essential to assess CR persistence and to verify the absence of tumoral invasion in the contralateral eye, which requires prompt treatment. Brain MRI should also be performed regularly to exclude secondary asymptomatic CNS involvement.

PERSPECTIVES AND CONCLUSIONS
In clinical practice, several therapeutic options are available, from local treatments to systemic chemotherapies and IC+ASCT, with practices that differ between ophthalmologists and oncologists/hematologists. IVT chemotherapy, mainly MTX and rituximab, or ORT can rapidly decrease vitreous disease involvement and improve vision but will not prevent the risk of CNS relapse. Local treatments still represent convenient symptomatic therapeutic options in patients with persistent PVRL who are unable to receive systemic treatment either because of their characteristics and comorbidities or the failure of systemic treatments. Although the lack of multi-institutional prospective studies prevents unequivocal conclusions on the superiority of one specific therapeutic approach, our own preference is to initially provide a PCNSL-like treatment with additional local ophthalmic treatment to younger patients with the objective of achieving a local CR and preventing CNS relapse. Nonetheless, the best combined treatment has not yet been defined. Encouraging results have been observed with single-agent oral temozolomide, ibrutinib and lenalidomide in patients with relapsed or refractory PVRL, supporting the need to assess these drugs as first-line treatments. Myeloablative chemotherapy followed by ASCT can be offered to patients up to 65 years of age and to a subgroup of selected older patients with R/R PVRL. The risks of each therapeutic option must be considered at the time of the therapeutic decision along with the patient’s characteristics. The results of a prospective study with single-agent pembrolizumab in patients with PCNSL, including a cohort of patients with PVRL, are pending (NCT03012620). Another major challenge in PVRL is the assessment of the therapeutic response. A prospective evaluation of the IL-10 level in the aqueous humor in addition to clinical and imaging ophthalmological examinations are currently the most promising feasible steps.
The diagnosis of PVRL remains challenging because of the fragility of the lymphoma cells in the vitreous but is being improved by recent advances in cytokine assays and molecular biology. International collaborative prospective studies bringing together ophthalmologists, biologists and oncologists/hematologists are necessary to improve the therapeutic outcomes and to address the issue of therapeutic response criteria for PVRL.
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<table>
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<tr>
<th>PVRL</th>
<th>Uveitis</th>
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<tbody>
<tr>
<td>Ocular injection</td>
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<tr>
<td>Anterior chamber inflammation</td>
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<tr>
<td>Choroid</td>
<td>No infiltration*</td>
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<td>Optical coherence tomography (OCT)</td>
<td>Subretinal deposits</td>
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<tr>
<td>Fluorescein angiography</td>
<td>Hypofluorescent spots (active lesions), window defects (scar), retinal pigment epithelium disturbances corresponding to a leopard skin pattern</td>
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<tr>
<td>Fluorescein angiography</td>
<td>Papillitis, vasculitis, cystoid macular edema, inflammatory capillaropathy, hypo or hyperfluorescent lesions, subretinal fluid, pinpoint</td>
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*Lymphomatous infiltration of the choroid is suggestive of a primary choroidal lymphoma (mainly MALT [mucosa-associated lymphoid tissue] lymphoma).
<table>
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<tr>
<th>Before sampling</th>
<th>Technique for sampling</th>
<th>Eye samples</th>
<th>Analysis of sample</th>
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| Discussion with the pathologist for prompt processing of the sample | Gentle diagnostic vitrectomy (low cutting speed) | Pure vitreous | Cytology
Immunocytochemistry, flow cytometry
Preferably without a fixative and processed within 2 hours after the reception. If a fixative is required: HOPE fixation is preferred

At least 4 cytospins
2 cytospots for cytomorphological evaluation after MGG coloration
2 cytospots for immunocytochemistry
Use the total amount of the available material

Cytokines (IL-10 and IL-6) without a fixative | Storage at -80°C
Measure of cytokine levels in thawed samples

Vitreous diluted in BSS during the surgical procedure | Preferably without a fixative and processed within 2 hours after the reception. If fixative is required: HOPE fixation | Immunocytochemistry, flow cytometry, molecular biology | 2 cytospots for cytomorphological evaluation after MGG coloration
2 cytospots for immunocytochemistry
Remaining slides stored at -30°C

HOPE: Herpes/glutamic acid buffer-mediated Organic solvent Protection Effect; MGG: May-Grünwald-Giemsa; BSS: Balanced Salt Solution

Adapted from Coupland et al. 24, Gonzales et al. 27 and Merle-Béral et al. 99.
Table 3. Available pharmacokinetic data of drugs in the ocular compartments

<table>
<thead>
<tr>
<th>Ref</th>
<th>Drug</th>
<th>Route</th>
<th>N</th>
<th>Dose</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bauma</td>
<td>Cytarabine</td>
<td>IV</td>
<td>79</td>
<td>3 g/m²</td>
<td>At 90 min after perfusion: C [vitreous] = 22µM; C [aqueous humor] = 31 µM; C [serum] = 11µM</td>
</tr>
<tr>
<td>Batchelor</td>
<td>Methotrexate*</td>
<td>IV</td>
<td>80</td>
<td>8 g/m³</td>
<td>At 4 hours after perfusion: C [vitreous] &gt; 1µM</td>
</tr>
<tr>
<td>Jahnke</td>
<td>Ifosfamide</td>
<td>IV</td>
<td>81</td>
<td>1.5-2 g/m²</td>
<td>At the end of perfusion: C [aqueous humor] = 0.32-1.56 µM in 6 patients</td>
</tr>
<tr>
<td></td>
<td>Trofosfamide</td>
<td>Oral</td>
<td>4</td>
<td>150-400 mg</td>
<td>C [aqueous humor] = 7.2 µM in one patient 4 hours after ingestion</td>
</tr>
<tr>
<td>De Smet</td>
<td>Methotrexate*</td>
<td>IVT</td>
<td>78</td>
<td>400 µg (4 mg/m³)</td>
<td>C [vitreous] ≈ 1µM 5 days after IVT</td>
</tr>
<tr>
<td>Kim</td>
<td>Rituximab</td>
<td>IVT</td>
<td>60</td>
<td>1 mg/0.1 ml</td>
<td>Half-life: 4.7 days; Clearance from the aqueous humor: 1.2 mL/min</td>
</tr>
</tbody>
</table>

IV: intravenous; C: concentration; IVT: intravitreal injection; * the minimal effective tumoricidal (cytotoxic) level is 1µM; ** 4-OH metabolites were dosed
### Characteristics and therapeutic results of retrospective and prospective studies of first-line treatment for primary vitreoretinal lymphoma

<table>
<thead>
<tr>
<th>Ref</th>
<th>Type of study Time span</th>
<th>N</th>
<th>Median age (range)</th>
<th>Type of treatment</th>
<th>Survival</th>
<th>CNS events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grimm(^9)</td>
<td>International multicenter retrospective study 1977-2005</td>
<td>83</td>
<td>63 (24-85)</td>
<td>Local (IVT MTX or ORT): N = 23 Systemic + local*: N = 53</td>
<td>Median PFS = 29.6 months Median OS = 58 months Median FU of surviving patients = 32 months No difference among treatment groups</td>
<td>CNS relapse in 29 patients (35%) No difference among treatment group</td>
</tr>
<tr>
<td>Riemens(^50)</td>
<td>European multicenter retrospective study 1991-2012</td>
<td>78</td>
<td>58 (39-86)</td>
<td>Local (IVT MTX ± Ritux or ORT): N = 31 Extensive: N = 21 Local and extensive *: N = 23</td>
<td>PFS: NA 5-y OS = 68% when no CNS relapse occurred 5-y OS = 35% when CNS relapse occurred</td>
<td>Median CNS-free survival = 47 months No difference between treatment groups</td>
</tr>
<tr>
<td>Klimova(^51)</td>
<td>Retrospective single-center study 2004-2016</td>
<td>10</td>
<td>59 (48-71)</td>
<td>Local (IVT MTX): N = 3 Local + IV MTX-based: N = 7</td>
<td>Median PFS not reached in the local + IV treatment group NA in the local treatment group P = 0.032</td>
<td>NA</td>
</tr>
</tbody>
</table>
| Castellino\(^51\) | Retrospective single-center study 1990-2018                  | 32*** | 67 (36-84)       | Local (IVT MTX ± Ritux or ORT): N = 17 IV\(^5\): N = 7 Local + IV: N = 8 | PFS: NA FFS:  
- Local: 1.8 y  
- IV: 3.2 y  
- Local + IV: NR P = 0.002 | Median CNS-free survival: NR CNS relapse = 37 % with a median FU = 2.8 y |
<p>| Cheah(^53) | Retrospective single-center study 2007-NA                    | 11 | 66 (48-72)         | Local + IV RMPV + 36 Gy binocular + HD-AraC | Median PFS = 3.8 y | 4-year cumulative incidence of CNS progression = 58% |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Dates</th>
<th>Patients</th>
<th>Treatment</th>
<th>Follow-up</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taoka</td>
<td>Retrospective single-center study</td>
<td>2007-2009</td>
<td>5</td>
<td>Local (IVT-MTX or ORT) + IV-RMPV + rdWBRT</td>
<td>65 (43-72)</td>
<td>5 patients alive in CR with a median FU = 32 months</td>
</tr>
<tr>
<td>Hashida</td>
<td>Retrospective single-center study</td>
<td>2001-2011</td>
<td>26</td>
<td>Local IVT-MTX or IVT-Ritux: N=15 Local + systemic IV-HDMTX or IT-MTX: N = 11</td>
<td>67 (36-85)</td>
<td>NA</td>
</tr>
<tr>
<td>De la Fuente</td>
<td>Retrospective single-center study</td>
<td>2005-2018</td>
<td>12</td>
<td>Local (Bilateral ORT) + MPV+/- R: N = 10 Bilateral ORT: n = 1 IVT-MTX: n = 1</td>
<td>64 (38-81)</td>
<td>5-y PFS = 62% 5-y OS = 71%</td>
</tr>
<tr>
<td>Akiyama</td>
<td>Prospective single-center cohort study</td>
<td>2007-2013</td>
<td>10</td>
<td>Local (IVT-MTX) followed by IV HD-MTX</td>
<td>68 (46–78)</td>
<td>NA</td>
</tr>
<tr>
<td>Kaburaki</td>
<td>Prospective single-center study</td>
<td>2008-2015</td>
<td>11</td>
<td>Local (IVT-MTX) + systemic IV-RMPV + rdWBRT† + IV-HD-AraC</td>
<td>63 (43-72)</td>
<td>4-y PFS = 73% 4-y OS = 89%</td>
</tr>
<tr>
<td>Lam</td>
<td>Retrospective multicenter study</td>
<td>2011-2018</td>
<td>59</td>
<td>Systemic IV-HDMTX-based chemotherapy: N = 51 Local (IVT-MTX or ORT) + systemic IV-HDMTX: N = 8</td>
<td>70 (39-88)</td>
<td>Median PFS = 18 months Median OS = 75 months</td>
</tr>
</tbody>
</table>
PVRL: primary vitreoretinal lymphoma; PFS: progression-free survival; FFS: failure free survival; OS: overall survival; CNS: central nervous system; MTX: methotrexate; IVT: intravitreal; IT: intrathecal; HD: high dose; IC + ASCT: intensive chemotherapy and autologous stem cell transplantation; IV: intravenous; Ritux: rituximab; ORT: ocular radiotherapy; NA: not available; ns: not significant; NR: not reached; *Systemic + local treatment included various combinations of systemic chemotherapy, ocular chemotherapy, whole brain radiotherapy, and ocular radiotherapy. Systemic chemotherapy was methotrexate-based therapy in 37 patients (70%). ** Local treatment included local radiotherapy and IVT MTX and/or rituximab. Extensive treatment regimens were heterogeneous and included HD MTX or aracytine (n = 40), whole-brain radiotherapy (n = 6), and peripheral blood stem cell transplantation (n = 4). *** In this paper, 33 patients had PVRL, 18 with concurrent intraocular and CNS or systemic disease and 18 with secondary VRL. One patient with PVRL did not receive any treatment. Only patients with PVRL and treated were considered in this review. $Detailed descriptions of the systemic treatments administered to the subgroup of patients with PVRL are not available. MPV: HD-MTX, procarbazine, vincristine; RMPV: rituximab, HD-MTX, procarbazine, vincristine; FU = follow-up; rdWBRT: reduced dose whole brain radiotherapy (23.4 Gy); †IV chemotherapy and rdWBRT were administered to patients who achieved a CR; AraC: cytarabine; NS: not significant
<table>
<thead>
<tr>
<th>Ref</th>
<th>Type of study</th>
<th>N</th>
<th>Median age (range)</th>
<th>Treatment</th>
<th>Responses</th>
<th>CNS relapse</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systemic treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubenstein\textsuperscript{92}</td>
<td>Prospective phase I trial</td>
<td>5*</td>
<td>66 (47-79)</td>
<td>Lenalidomide (10-30 mg/day 21 days/28)</td>
<td>1 CR; 3 PR; 1 SD</td>
<td>NA</td>
<td>PFS: 1.75, 6, 9, 21 and 48+</td>
</tr>
<tr>
<td>Ghesquieres\textsuperscript{89}</td>
<td>Prospective multicenter phase II trial</td>
<td>17**</td>
<td>69 (46-86)</td>
<td>Ritux iv (375 mg/m\textsuperscript{2}) Lenalidomide (20-25 mg/day 21 days/28)</td>
<td>CR = 35%</td>
<td>NA</td>
<td>mFU = 19 months</td>
</tr>
<tr>
<td>Soussain\textsuperscript{80}</td>
<td>Prospective multicenter phase II trial</td>
<td>14**</td>
<td>67 (47-82)</td>
<td>Ibrutinib (560 mg/day)</td>
<td>CR = 50%</td>
<td>N = 1 (mFU = 26 months)</td>
<td>Median PFS = 23 months</td>
</tr>
<tr>
<td>Baron\textsuperscript{88}</td>
<td>Retrospective multicenter study</td>
<td>21</td>
<td>75 (35-90)</td>
<td>Temozolomide (150 mg/m\textsuperscript{2}/day x 5)</td>
<td>CR = 71%</td>
<td>N = 5 (mFU = 42 months)</td>
<td>PFS = 12 months</td>
</tr>
<tr>
<td>Jahnke\textsuperscript{100}</td>
<td>Retrospective study</td>
<td>10</td>
<td>NA</td>
<td>Ifosfamide IV or trofosfamide PO</td>
<td>CR: N = 9 PR: N = 1</td>
<td>N = 2</td>
<td>PFS = 18 m OS = 32 m</td>
</tr>
<tr>
<td>Soussain\textsuperscript{83 †}</td>
<td>Retrospective study</td>
<td>5</td>
<td>NA</td>
<td>TBC + ASCT</td>
<td>CR: N = 5 PR: N = 0</td>
<td>N = 0</td>
<td>3 patients alive at 14, 14, 15 months</td>
</tr>
<tr>
<td>Soussain\textsuperscript{84 †}</td>
<td>Retrospective study</td>
<td>11*</td>
<td>53 (27-64)</td>
<td>TBC + ASCT</td>
<td>CR: N = 9 PR: N = 2</td>
<td>N = 0</td>
<td>7 patients alive with a CR at 18, 28, 41, 45, 49, 62, 69, and 70 months</td>
</tr>
<tr>
<td>Soussain\textsuperscript{85}</td>
<td>Retrospective study</td>
<td>11*</td>
<td>52 (23-67)</td>
<td>TBC + ASCT</td>
<td>NA for the PVRL subgroup of patients</td>
<td>5-y PFS = 50% (no significant difference between patients with PVRL and PCNSL)</td>
<td></td>
</tr>
</tbody>
</table>

CNS: central nervous system; IV: intravenous; PO: oral route; CR: complete response; PR: partial response; PFS: progression-free survival; OS: overall survival; TBC: thiotepa (750 mg/m\textsuperscript{2})-busulfan (8 mg/kg)-cyclophosphamide (120 mg/kg); ASCT: autologous stem cell transplantation; mFU: median follow-up; iv: intravenous
†: These two studies did not include the same patients; *: number of patients with intraocular lymphoma (isolated intraocular relapse of a primary CNS lymphoma n = 3; intraocular and brain relapse of a systemic lymphoma n = 1; intraocular and brain relapse of a primary CNS lymphoma n = 1); **number of patients included in the studies with isolated intraocular disease involvement (R/R PVRL, and intraocular relapse of PCNSL)
NA: not available
Figure 1. Schema of the anatomy of the eye and anatomical classification of intraocular lymphomas (IOLs).

IOLs can occur in the uveal tract (iris, ciliary body and choroid) as a primary (mainly MALT [mucosa-associated lymphoid tissue] lymphoma) or secondary (to systemic lymphoma) form.

IOLs that occur into the vitreous body, the retina or in rare cases the optic nerve are called vitreoretinal lymphomas (VRLs). VRLs are subdivided into primary vitreoretinal lymphomas (PVRLs), ocular involvement of primary central nervous system lymphoma (PCNSL) and secondary lymphoma from a systemic diffuse large B-cell lymphoma (DLBCL).

The typical localization of lymphoma cells in the case of PVRL is represented by the small yellow circles drawn on the picture. Image A: Slit-lamp examination of an eye affected by PVRL showing the infiltration of lymphoma cells in the anterior vitreous and along the vitreous fibrils. Image B: Optical coherence tomography (OCT) image of the retina illustrating the infiltration of lymphoma cells into the subretinal space between the retinal pigmented epithelium (yellow arrow) and the Bruch’s membrane (white arrow) or into the vitreous body.

Figure 2. Ophthalmological findings and multimodal imaging of primary vitreoretinal lymphoma. A: Slit-lamp examination showing a quiet anterior chamber with no protein flare and some cellular gray diffuse keratic precipitates (white arrows). B: A clump of lymphoma cells is visible in the slit-lamp examination in the anterior vitreous or along the vitreous fibrils (yellow arrow). C: At diagnosis, a fundus examination reveals only subtle small yellowish retinal lesions (white arrow). D: The (sub)retinal lesions detected at diagnosis are more extended and multifocal (yellow arrow) (composite color fundus photograph, photos merged together with built-in camera software) E: Retinal photograph showing cream-colored subretinal deposits (white stars). F: Infrared photograph of the same patient illustrated in E, showing hyperreflective lesions corresponding to the lesions visible in E (white stars). G: Fluorescein angiography of the same patient illustrated in E, showing the lymphoid infiltration as dark spots masking the underlying fluorescence of the choroid (white stars). Retinal pigmented epithelium (RPE) alterations appear as hyperfluorescent areas due to window effects (yellow arrow). H: Indocyanine angiography of the same patient illustrated in E, poorly contributive; hypocyanescent lesions are visualized. I: Fundus picture showing patchy yellowish subretinal lesions (white star). J: OCT image of the retina along the white line in I showing subretinal hyperreflective deposits of lymphoma cells (white star) that are located between the RPE (yellow arrow) and the Bruch membrane (white arrow).

Figure 3. Typical cytology of vitreoretinal lymphoma. Reconstruction from 4 fields of the same cytospin.

A: May-Grünvald-Giemsa staining of a diluted vitrectomy specimen. Cytospin shows 2 large lymphoid cells with a basophilic cytoplasm and irregular nuclei with several nucleoli corresponding to large lymphomatous cells. A small reactive lymphocyte (insert picture) was visible on another field of the same cytospin.

B: Immunochemistry with an anti-CD3 antibody and APAAP staining showing the negativity of a large lymphoma cell and the positivity of a reactive lymphocyte (inset picture). These two cells were identified in two different fields on the same cytospin.
C: Immunochemistry with an anti-CD20 antibody and APAAP (Alkaline Phosphatase-Anti-Alkaline Phosphatase) staining showing the positivity of a large lymphoma cell and the negativity of a reactive lymphocyte (inset picture). These two cells were identified in two different fields on the same cytospin.

Figure 4. Interleukin levels in case of a clinical suspicion of primary vitreoretinal lymphoma. A. IL-10 (interleukin 10) and IL-6 levels in the aqueous humor and vitreous for PVRL screening and diagnosis. In the aqueous humor, an absolute level of IL-10 ≥50 pg/mL and IL-10/IL-6 ratio greater than 1 is suggestive of PVRL (IL-10 cutoff: sensitivity 89%, specificity 93%). In patients with uveitis, the level of IL-10 is low and IL-10/IL-6 ratio is lower than 1. In patients with an infectious disease, the IL-10 level might exceed 50 pg/mL, but the IL-6 level might also be higher than 50 pg/mL: the IL-10/IL-6 ratio is lower than 1. In the vitreous, the absolute level of IL-10 ≥400 pg/mL and IL-10/IL-6 ratio greater than 1 are also suggestive of PVRL (IL-10 cutoff: sensitivity 80%, specificity 99%). From Cassoux et al. Investigative Ophthalmology and Visual Science, 2007; Chan et al. American Journal of Ophthalmology, 1995.

B. ISOLD score. The Interleukin Score for intraOcular Lymphoma Diagnosis (ISOLD) score is a probability score for the diagnosis of PVRL based on a mathematic formula combining both IL-10 and IL-6 levels in vitreous or aqueous samples with high sensitivity and specificity (93% and 95% respectively). From Costopoulos et al., Ophthalmology, 2016.

Figure 5. Clinical evaluation of the response to treatment of primary vitreoretinal lymphoma. A: Retinal photograph of a subretinal yellowish lesion corresponding to the lymphoma lesion before treatment (white arrow). B: OCT (Optical Coherence Tomography) image of the lesion described in A showing hyperreflective subretinal deposits (white star). C: After systemic treatment, the subretinal lesion completely disappeared in the retinal photograph (yellow arrow). D: In the OCT image, the subretinal deposits also disappeared (yellow star). E: In another patient, composite color fundus photograph (photos merged together with built-in camera software) shows a diffuse subretinal yellowish lesions corresponding to (sub)retinal infiltration by lymphoma cells captured before treatment (white arrow). In this patient, the vitreous was clear and free of visible lymphoma cells. The vitreous levels of IL-10 and IL-6 were low (36 pg/mL and 6 pg/mL respectively). A retinal biopsy was performed in the area of the white star to assess the diagnosis in this patient with pure retinal lymphoma. F: composite color fundus photograph (photos merged together with built-in camera software) of patient illustrated in E, after two courses of systemic chemotherapy (rituximab, high-dose methotrexate, procarbazine, and vincristine), showing the disappearance of most of the lesions (yellow arrow). The residual active lesions (blue arrows) disappeared after subsequent courses of chemotherapy. The scar from the retinal biopsy performed during diagnostic vitrectomy is visible in the nasal side of the optic nerve (yellow star).
A. IL dosage in aqueous from anterior chamber tap

- IL-10 ≥50 pg/ml
- IL-10 < 50 pg/ml
- IL-10/IL-6 ratio > 1
- IL-10/IL-6 ratio < 1

Highly suggestive of PVRL
Suggestive of infectious disease
Suggestive of inflammatory uveitis

IL dosage in vitreous from diagnostic vitrectomy

- IL-10 ≥400 pg/ml and IL-10/IL-6 ratio > 1
- Highly suggestive of PVRL

B. ISOLD score

ISOLD formula for aqueous: \(-12.871 + 5.533 \times \log(\text{IL-10} + 1) - 1.614 \times \log(\text{IL-6} + 1)\).
ISOLD formula for vitreous: \(-12.208 + 4.648 \times \log(\text{IL-10} + 1) - 1.669 \times \log(\text{IL-6} + 1)\).
Each ISOLD value is associated with a probability calculated using the following function:

\[
\text{Probability (intraocular lymphoma)} = \frac{1}{1 + \exp(-\text{ISOLD})}
\]