**How we diagnose and treat intraocular lymphoma?**

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**Abstract**

The eye is a rare site for the development of malignant lymphoma. Based on cell type and involved intraocular structures, which as a whole represent an immune-privileged site, several subtypes of primary intraocular lymphoma need to be discerned. Primary vitreoretinal lymphoma (PVRL), the most common form, is an aggressive B-cell malignancy and considered a subtype of primary CNS lymphoma. Diagnosis of PVRL is usually based on the analysis of vitreous biopsy material. In addition to cytological and immunocytochemical examination, measurements of cytokine levels and molecular determination of B-cell clonality and recurrent mutations increase the diagnostic yield. [AJMF: Please include a few words on presentation, natural behavior and prognosis]Both systemic chemotherapy and exclusively local treatment, including ocular radiotherapy and intravitreal chemotherapy, are successful approaches for the management of PRVL, although it is currently not predictable which patients require systemic treatment in order to avoid cerebral dissemination [AJMF: This should be better explained - it depends (linked to) of the sentence on natural behavior].

Key: primary CNS lymphoma, intraocular lymphoma, vitreoretinal lymphoma.

**Introduction**

Malignant lymphomas involving the eye and surrounding structures are rare, representing less than 10% of extranodal lymphoma. Due to the unique anatomical situation, the limitations for invasive procedures and the desire to preserve vision, both diagnosis and treatment of intraocular lymphoma pose specific challenges and require close collaboration between ophthalmologists, pathologists and oncologists. Based on clinical and pathological features and topography, two groups need to be discerned, namely lymphomas involving the ocular adnexae and the orbit, which mainly include extranodal marginal zone B-cell lymphoma of MALT type and involvement by different types of systemic non-Hodgkin’s lymphoma, and those affecting intraocular structures, including the retina and vitreous body, the uvea, iris and anterior segments of the eye, which are almost invariably aggressive lymphoma entities ([Coupland and Damato 2008](#_ENREF_20), [Coupland*, et al* 2004](#_ENREF_22)). The latter group will be covered in this review. [AJMF: The concept of “primary” and “secondary” forms should be clearly stated in the first paragraph]

The term ‘intraocular lymphoma’ was first used more than 60 years ago ([Cooper and Riker 1951](#_ENREF_15), [Qualman*, et al* 1983](#_ENREF_58)) but later replaced with the now obsolete term ‘ocular reticulum cell sarcoma’. Since there are great differences in the biology, clinical behavior and prognosis depending on the affected anatomical compartment of the eye and neoplastic cell type, the term ‘intraocular lymphoma’ should be replaced by a more specific terminology reflecting both the precise location, as well as the histological subtype of lymphoma ([Coupland*, et al* 2009](#_ENREF_19)). The by far most common primary intraocular lymphoma is primary vitreoretinal lymphoma (PVRL), an aggressive disease classified as diffuse large B-cell lymphoma (DLBCL) and regarded as a manifestation of primary central nervous system lymphoma (PCNSL), reflecting the common embryological origin of the two organs ([Kluin*, et al* 2008](#_ENREF_42)). Given the more common occurrence of PCNSL, much of the current state of knowledge about biology and clinical behavior of VRL is actually inferred from the investigation of PCNSL. This review is a critical analysis of the current state of the art for the diagnosis and treatment of VRL and discuss available literature on other lymphoma entities that may infiltrate the eye.

**Classification of intraocular lymphoma**

Based on anatomical compartment and neoplastic cell type, several types of **primary** intraocular lymphoma with distinct distribution patterns, histology and behavior can be discerned ([Coupland*, et al* 2009](#_ENREF_19), [Coupland and Damato 2008](#_ENREF_20)). Their salient clinical and pathological features are summarized in Table 1. **Secondary** lymphomatous involvement of intraocular structures represents either an extension of PCNSL and affects the vitreoretinal compartment, identical to primary VRL, or less commonly a secondary manifestation of a systemic Non-Hodgkin lymphoma (NHL), typically affecting the uvea (i.e. iris, ciliary body or choroid). [AJMF: This paragraph should be moved as first paragraph in the introduction section]

**Biological and pathological features of PVRL**

PVRL involving the retina, the vitreous or both structures is by far the most common form of primary intraocular lymphoma. Involvement of the CNS is common, occurs in 16-34% of cases at presentation and develops in 35-90% during the course of the disease. Conversely, approximately 15-25% of patients with PCNSL develop VRL ([Grimm*, et al* 2008](#_ENREF_36), [Grimm*, et al* 2007](#_ENREF_37)). In contrast to systemic DLBCL and similar to PCNSL, extracerebral dissemination is very rare. The reasons for this are unclear: some immunophenotypical studies have shown distinct patterns of chemokine and chemokine receptor expression on the malignant cells of PCNSL, which might explain their dissemination characteristics. Interaction of B-cell chemokines CXCL12 and CXCL13 with their receptors CXCR4 and CXCR5 mediates chemotaxis and may promote B-cell survival ([Chan*, et al* 2003](#_ENREF_13), [Deckert*, et al* 2014](#_ENREF_27), [Montesinos-Rongen*, et al* 2008](#_ENREF_50), [Smith*, et al* 2003](#_ENREF_65), [Smith*, et al* 2007](#_ENREF_66)). Elevated levels of CXCL13 in cerebrospinal fluid (CSF) have been associated with poor prognosis and may be used for diagnostic purposes ([Rubenstein*, et al* 2013](#_ENREF_62)). However, studies confirming these site-specific expression profiles and their potential impact on clinical behavior of VRL are lacking.

Furthermore, the eye, similar to the brain and the testis represents an immune-privileged site in which normal mechanisms of immune recognition of foreign antigen and immune-mediated inflammation are inactive [ref]. The absence of local immune surveillance might result in acquisition of distinct biological features by the neoplastic cells, which in turn could prevent their efficient dissemination to extracerebral sites. However, manifest VRL often contains an abundance of reactive T-cells and macrophages, indicating that the development of clinically manifest lymphoma is associated with a breakdown of the immune-privileged state. Of note, PCNSL as well as DLBCL arising in the testis, another immune-privileged site, frequently show lack of HLA class I and II expression, due to deletions of 6p21.32 harboring the HLA locus, which may allow escape from immune attack ([Riemersma*, et al* 2000](#_ENREF_61)).

*Cell of origin and immunophenotype of PVRL*

The vast majority (~95%) of PVRL can be classified as diffuse large B-cell lymphoma (DLBCL) (Figure 1); however, due to their unique clinical and biological features, PCNSL including PVRL, is recognized as a specific subtype of lymphoma in the WHO classification ([Kluin*, et al* 2008](#_ENREF_42)). Primary DLBCL of the CNS (including VRL) belong to the activated B-cell (ABC) type of DLBCL according to gene expression profile and mutational status in 80-90% of cases ([Montesinos-Rongen*, et al* 2008](#_ENREF_50)). In addition to pan-B-cell markers such as CD20, Pax-5 and CD79a, they therefore express MUM1/IRF4, commonly BCL-6 and BCL-2 and usually lack CD10 and plasma cell markers, showing that biologically they are arrested at a late germinal center B-cell differentiation stage ([Camilleri-Broet*, et al* 2006](#_ENREF_9), [Coupland*, et al* 2005c](#_ENREF_24)). VRL usually expresses monotypic immunoglobulin light chains and IgM or IgM and IgD heavy chains ([Coupland*, et al* 2009](#_ENREF_19)). Their proliferation and apoptotic rate is high, also reflected in commonly high numbers of necrotic cells. As B-cells having passed through the germinal center reaction, VRL commonly show a high rate of somatic mutation of the rearranged immunoglobulin genes, in most cases without evidence for ongoing hypermutation, and similar to PCNSL frequently exhibit immunoglobulin rearrangements using VH 4-34 ([Coupland*, et al* 2005b](#_ENREF_23), [Malumbres*, et al* 2007](#_ENREF_46), [Montesinos-Rongen*, et al* 1999](#_ENREF_52)).

*Genetics of PVRL*

Genetic studies of VRL are sparse, and most published data stem from studies of PCNSL. Earlier studies of VRL using PCR have demonstrated a high frequency of IGH/BCL2 rearrangements as a result of the t(14;18) translocation, which occur in 85-90% of follicular lymphoma and in about 30% of DLBCL of germinal center B-cell type ([Chan 2003](#_ENREF_11), [Wallace*, et al* 2006](#_ENREF_73)). These results, however, are somehow at odds with the notion that most PVRL are of ABC type, which lacks BCL2 rearrangements, and with the fact that BCL2 translocations are rare in PCNSL ([Montesinos-Rongen*, et al* 2002](#_ENREF_53)). Translocations involving the BCL-6 oncogene occur in 17-47% of PCNSL, and activation of this master regulator of the germinal center reaction may in part be responsible for the arrest in the terminal B-cell differentiation stage ([Cady*, et al* 2008](#_ENREF_8), [Montesinos-Rongen*, et al* 2002](#_ENREF_53)). Their presence in VRL has not been studied to date. Using a high resolution SNP array for the identification of copy number changes, large numbers of alterations with common gains on chromosomes 1q, 18q and 19q and frequent losses on 6q, alterations which are also frequently identified in PCNSL, have been demonstrated in VRL ([Wang*, et al* 2014](#_ENREF_74)).

Recently, several studies using conventional techniques or next generation sequencing have analyzed the mutational landscape of PCNSL and have identified high frequencies of mutations in Myeloid Differentiation Factor 88 (*MYD88*), a member of the toll-like receptor pathway, and members of the B-cell receptor signaling pathway including *CD79b*, as well as other genes resulting in a constitutive activation of NF-kB signaling ([Bonzheim*, et al* 2015](#_ENREF_5), [Braggio*, et al* 2015](#_ENREF_6), [Bruno*, et al* 2014](#_ENREF_7), [Gonzalez-Aguilar*, et al* 2012](#_ENREF_35), [Kraan*, et al* 2013](#_ENREF_43), [Nakamura*, et al* 2015](#_ENREF_55)). Furthermore, data generated by whole exome sequencing suggest a major impact of aberrant somatic hypermutation (SHM) on the mutational profile of PCNSL. SHM is the process by which mutations are introduced into the rearranged immunoglobulin genes of germinal center B-cells in order to increase the binding affinity of the B-cell receptor. In addition to well-known targets, such as *C-MYC, PAX-5* and *PIM*, aberrant SHM seems to target additional genes in PCNSL, some of them involved in CNS development ([Vater*, et al* 2015](#_ENREF_71)).

Mutations affecting *MYD88,* most commonly the canonical L265P mutation, also found in the vast majority of lymphoplasmacytic lymphomas/Waldenström’s macroglobulinemia, and CD79b, although also present in DLBCL of ABC type of other locations, seem to be enriched specifically in DLBCL of immune-privileged sites, namely the testis and the CNS. *MYD88* mutations have been found in 35-79% of PCNSL ([Braggio*, et al* 2015](#_ENREF_3), [Gonzalez-Aguilar*, et al* 2012](#_ENREF_25), [Kraan*, et al* 2013](#_ENREF_32), [Montesinos-Rongen*, et al* 2011](#_ENREF_38), [Nakamura*, et al* 2015](#_ENREF_41)) and were recently identified in 69% of VRL with or without concomitant cerebral involvement ([Bonzheim*, et al* 2015](#_ENREF_5), [Braggio*, et al* 2015](#_ENREF_6), [Gonzalez-Aguilar*, et al* 2012](#_ENREF_35), [Kraan*, et al* 2013](#_ENREF_43), [Montesinos-Rongen*, et al* 2011](#_ENREF_51), [Nakamura*, et al* 2015](#_ENREF_55)).

Very little is known so far about epigenetic alterations and micro-RNA (miRNA) expression profiles in PVRL, and only few studies have addressed these topics in PCNSL ([Deckert*, et al* 2014](#_ENREF_27)). Of interest, a recent study has compared miRNA expression profiles in vitreal aspirates of PVRL and uveitis, and identified miRNA-155 as consistently differentially expressed and as potential novel biomarker ([Tuo*, et al* 2014](#_ENREF_69)); however, further validation studies are needed to confirm the value of miRNA analysis for PVRL diagnosis.

*VRL in immunosuppressed patients*

Epstein-Barr virus (EBV) is identified at a very high frequency in immunosuppressed patients with PCNSL, especially in the setting of AIDS ([MacMahon*, et al* 1991](#_ENREF_45)), but absent from lymphomas in immunocompetent patients. The same seems true for patients with VRL, although the number of cases studied for the presence of EBV is low ([Chan 2003](#_ENREF_11)).

*Other types of primary and secondary intraocular lymphoma*

As outlined in Table 1, primary choroidal and ciliary body lymphoma usually belongs to the category of extranodal marginal zone lymphoma (MALT-type lymphoma), and may be associated with MALT-type lymphoma secondarily involving the ocular adnexae ([Coupland and Damato 2008](#_ENREF_20)). Separation from PVRL is important, because these lymphomas run an indolent course and lack involvement of the CNS. Patients with primary choroidal lymphoma have a very good prognosis (Figure 2). Other NHL subtypes including T-cell lymphoma with primary intraocular manifestation mostly have been reported as single case reports ([Coupland*, et al* 2005a](#_ENREF_17), [Ponzoni*, et al* 2002](#_ENREF_57)). Intraocular T-NHL seems to be associated with a less aggressive course, with or without CNS involvement ([Coupland and Damato 2008](#_ENREF_20)).

Secondary intraocular involvement by systemic lymphoma is relatively rare, and the disease is usually confined to the choroid. The most common subtype is systemic DLBCL ([Coupland and Damato 2008](#_ENREF_20)) but secondary infiltration of the eye by other B-NHL subtypes include chronic lymphocytic leukemia ([Coupland*, et al* 2001](#_ENREF_21)), plasma cell neoplasms ([Fung*, et al* 2005](#_ENREF_34)), as well as intravascular large B-cell lymphoma ([Mudhar*, et al* 2007](#_ENREF_54)). A peculiar phenomenon is the increased risk for secondary vitreoretinal or CNS involvement by DLBCL of the testis, another immune- privileged site, which shares many features with PVRL and PCNSL, including predominance of the ABC type, common loss of HLA class I and II expression and common *MYD88* mutations ([Kraan*, et al* 2013](#_ENREF_43), [Riemersma*, et al* 2000](#_ENREF_61)). Of interest, joint *MYD88* L265P mutations indicating a common clonal origin were identified in two cases of VRL with a history of testicular lymphoma in our recent VRL series ([Bonzheim*, et al* 2015](#_ENREF_5)).

**Epidemiology**

The true incidence of PVRL is unknown because no central database exists yet for this rare disease: hopefully, this major deficiency will be overcome soon through efforts of ‘The AJCC Ophthalmic Oncology Task Force’, who are presently establishing an international multicenter VRL registry. It can be confidently stated that PVRL is one of the rarest primary ocular tumors: a 20-year retrospective study at a large Canadian hospital estimated the incidence of VRL in British Columbia to be between 0.017-0.048/100,000 people during the years 1990 and 2010 ([Levasseur*, et al* 2013](#_ENREF_44)). Better data exist for PCNSL: the Central Brain Tumor Registry of the United States published the incidence of PCNSL in the U.S. as 0.46 per 100,000 person-years between 2004-2007 and 0.45 per 100,000 person-years between 2005-2009. The incidence was higher in males (0.54) than in females (0.39), with a male:female ratio of 1.38. Similar incidence rates have been published in Europe ([Hoang-Xuan*, et al* 2015](#_ENREF_39), [Phillips*, et al* 2014](#_ENREF_56)).

PVRL usually occurs in adults from the third to the eighth decades of life, with the median age at diagnosis in these patients being 63 years, without a gender prevalence ([Grimm*, et al* 2007](#_ENREF_37)). Whilst the most important risk factors for PCNSL are the HIV status and EBV infection status ([Phillips*, et al* 2014](#_ENREF_56)), there are no other known risk factors for PVRL.

**Clinical features of IOL**

Clinical presentation of IOL varies largely according to the involved ocular structures and to the presence or absence of concomitant brain and/or meningeal disease. Ocular symptoms are usually the only expression of disease in patients with PVRL. Most of these patients have good performance status, with a history of a prior malignancy in 10-15% of cases ([Hoang-Xuan*, et al* 2015](#_ENREF_39)).

The mean duration of PVRL symptoms prior to diagnosis is 6 months, but, in some cases, symptoms precede diagnosis of 2-3 years. The most common presenting symptoms are non-specific, and include blurred vision in 40-50% of patients, decreased visual acuity in 25-30% and floaters in 20-25%. Signs and symptoms of PRVL may mimic other intraocular conditions such as uveitis, thus making PVRL a “masquerade” syndrome ([AlQahtani*, et al* 2014](#_ENREF_2)). Bilateral ocular involvement is common, occurring in 60-90% of patients, but may appear clinically as unilateral involvement due to uneven distribution of disease. CSF dissemination is detected during staging workup in only 10-15% of cases ([Grimm*, et al* 2007](#_ENREF_37)).

Patients’ characteristics and clinical presentation do not differ greatly between patients with PVRL and patients with concomitant brain and ocular lymphoma. Median age and gender distribution are the same, whereas performance status is usually poorer in the latter group ([Hoang-Xuan*, et al* 2015](#_ENREF_39)). Ocular symptoms are similar to those above reported for PVRL patients, and can be concomitant to other neurological symptoms or precede the onset of brain lesions by weeks or months, and sometimes years. The most commonly associated neurological symptoms, which often span weeks to months, are focal deficits, personality changes and increased intracranial pressure: behavioral/cognitive changes in 25-30% of cases, hemiparesis in 10-15%, headache in 10-15%, aphasia in 10-15%, seizure in 5%, ataxia in 4% ([Ferreri*, et al* 2002](#_ENREF_30), [Hoang-Xuan*, et al* 2015](#_ENREF_39)). Patients with ocular symptoms and asymptomatic brain disease are uncommon (3% of cases) ([Grimm*, et al* 2008](#_ENREF_36)). The average duration of symptoms prior to diagnosis is usually shorter than that reported for PVRL (3 months). CSF infiltration is detected in 20-25% during staging in patents with concomitant ocular and cerebral disease.

**Diagnosis of VRL**

Ophthalmological examination in VRL frequently demonstrates the presence of vitritis, usually in association with infiltrates of the retina and the retinal pigment epithelium, sometimes giving the characteristic “leopard skin” pigmentation (Figure 1) on fundoscopy and fluorescein angiography ([Fardeau*, et al* 2009](#_ENREF_28)), whereas alterations in the anterior segment of the eye are usually absent ([Chan and Sen 2013](#_ENREF_12), [Coupland and Damato 2008](#_ENREF_20), [Sagoo*, et al* 2014](#_ENREF_63)). Although clinical examination and imaging procedures often lead to a high suspicion of lymphoma, VRL frequently mimics chronic posterior uveitis, including an initial response to steroids. As a classical “masquerade syndrome”, VRL requires diagnostic confirmation through invasive procedures providing morphological, phenotypical and/or molecular evidence for malignancy. In cases with concomitant CNS involvement, a positive CSF examination or stereotactic brain biopsy may obviate the need for intraocular biopsy. The standard approach to diagnosis of PVRL is vitrectomy or vitreous aspirate biopsy. In case this approach does not render a diagnosis, subretinal aspirate biopsy or chorioretinal biopsy or of other intraocular structures may be used ([Coupland 2012](#_ENREF_16)). For a detailed review of the surgical procedures, the reader is referred to the ophthalmological literature. The aspirated material can be used for cytological examination, immunocytochemistry, molecular examinations and determination of cytokine levels. Therefore, an adequate triage system to maximize the use of the limited material for the different techniques should be in place in the pathology laboratory, which ideally should be experienced in handling these samples. Pre-analytical conditions are of major importance. Cytological material should either be worked within an hour after aspiration, or alternatively put either into culture medium or a mild fixative such as HOPE solution which preserves cytological detail, as well as immunoreactivity and nucleic acids ([Coupland 2012](#_ENREF_16)). Cytological specimens are usually prepared with the cytospin technique; for cell-rich specimens, the cell-block technique can be employed alternatively.

*Cytology and immunocytochemistry*

Cytological examination reveals the presence of large, atypical lymphoid cells with increased, nuclear/cytoplasmic ratio, basophilic cytoplasm and irregular nuclei with one to several nucleoli in cases of VRL. However, large numbers of reactive lymphocytes, poor preservation of cytological detail due to degenerative changes and necrosis, and paucicellular aspirates due to limited involvement of the vitreous or an antecedent steroid therapy often preclude a diagnosis of malignancy based only on morphology. Furthermore, atypical large cells may also occur in reactive conditions, such as acute viral infection. The reported rates of sensitivity and specificity of cytology for the diagnosis of VRL vary widely, but cytology alone is able to confirm VRL in 45-60% of cases, and false positive results are considered rare ([Davis*, et al* 2005](#_ENREF_25), [Kimura*, et al* 2012](#_ENREF_41), [Wittenberg*, et al* 2008](#_ENREF_76)).

Immunocytochemistry is a valuable tool for confirming a diagnosis of VRL, with a predominance of large cells expressing pan B-cell markers such as CD20, PAX5 and CD79a. Alternatively, multicolor flow cytometry has been employed successfully for phenotyping of vitreal aspirates, with a reported sensitivity of 82% and 100% specificity ([Missotten*, et al* 2013](#_ENREF_49)). Again, however, poor cellular preservation and abundant reactive T-cells may limit the diagnostic yield ([Davis*, et al* 2012](#_ENREF_26)).

*Molecular diagnosis of VRL*

Molecular examination of vitreous specimens is a valuable tool to confirm a diagnosis of lymphoma, and the application of modern PCR techniques has reduced the necessary amount of material considerably. Identification of clonal immunoglobulin gene rearrangements using consensus primer sets such as those developed by the BIOMED-2 consortium is a mainstay in VRL diagnosis. The sensitivity of clonality studies ranges between 65-95%, depending on the choice of primer sets and quality of material ([Baehring*, et al* 2005](#_ENREF_3), [Coupland*, et al* 2003](#_ENREF_18), [Coupland*, et al* 2005b](#_ENREF_23), [Kimura*, et al* 2012](#_ENREF_41), [Merle-Beral*, et al* 2004](#_ENREF_48), [Wang*, et al* 2011](#_ENREF_75)). Depending on the number of primer sets used – which can be limited by the available material - VRL of DLBCL type may also yield false negative results due to somatic hypermutation abrogating primer binding. On the other hand, due to the unique situation of the eye as immune-privileged site, inflammatory conditions can also result in oligoclonal or even clonal expansions of lymphocytes and may lead to false positive results, especially in cases with low cellularity ([Bonzheim*, et al* 2015](#_ENREF_5), [Sugita*, et al* 2009](#_ENREF_68)). In order to avoid misdiagnosis of minor clonal expansions as evidence for lymphoma, all tests should be run in duplicate to confirm the presence of a dominant clone, and results should be interpreted with caution and only in the context of clinical and morphological findings. Determination of clonality is especially valuable for cases, in which suspected intraocular dissemination or relapse of PCNSL or systemic lymphoma can be confirmed by proving or disproving clonal relationship.

In order to increase the diagnostic yield of vitreous specimens, we recently have made use of the common occurrence of mutations of *MYD88* in PCNSL, identified in 50-70% of cases. Arguing that VRL as a subtype of PCNSL should show a similar high frequency, we used a sensitive allele-specific PCR for the most common mutation *MYD88* L265P, and conventional sequencing for exons 3 and 4 in a large series of archival vitreous specimens from two institutions ([Bonzheim*, et al* 2015](#_ENREF_5)). We detected *MYD88* mutations, in 20/28 samples of confirmed VRL, with the canonical L265P in all but a single case (Figure 1). None of the cases classified as reactive was positive for *MYD88* mutations. Importantly, this approach confirmed a diagnosis of VRL in 6 cases initially diagnosed as either only suspicious for lymphoma or reactive based on cytology, immunocytochemistry and clonality analysis, thus increasing the sensitivity of vitreous biopsy for VRL diagnosis from 62% to 90%. Although these findings need to be confirmed in a prospective manner, mutational analysis using sensitive techniques such as allele-specific PCR or next generation sequencing using mutation-specific panels likely will provide a valuable additional tool for diagnosis and perhaps follow-up of VRL.

*Determination of cytokine levels*

Some centers advocate the use of measurement of cytokine levels within ocular fluids – i.e. aqueous humor and the vitreous – to provide diagnostic evidence in addition to cytology for the presence/absence of PVRL ([Cassoux*, et al* 2007](#_ENREF_10), [Chan*, et al* 1995](#_ENREF_14), [Fisson*, et al* 2013](#_ENREF_32), [Mehta*, et al* 2015](#_ENREF_47), [Merle-Beral*, et al* 2004](#_ENREF_48), [Raja*, et al* 2013](#_ENREF_59), [Saleh*, et al* 2012](#_ENREF_64)). In particular, these centers measure the interleukin (IL) 10 and 6 levels and then compare their ratio: a high level of IL-10 in pure vitreous or aqueous humor samples, or an IL-10/IL-6 ratio greater than 1 in diluted or undiluted samples, is considered indirect evidence supporting the diagnosis of PVRL. The techniques used to measure the IL-10 and IL-6 levels include enzyme-linked immunosorbent assay (ELISA) and multiplex-based cytometric bead array. The exact cutoff for the IL-10 concentration or IL-10/IL-6 ratio may vary between laboratories, mainly due to differences in the methods applied, the conditions of sample harvesting and storage, techniques, and manufacturers of equipment and supplies, as well as the dilution (known or unknown) of the vitreous samples and the laboratory's own experience. Interleukin levels within intraocular fluids have also been proposed and used with some success to monitor response of PVRL under therapy ([Raja*, et al* 2013](#_ENREF_59), [Saleh*, et al* 2012](#_ENREF_64)). [AJMF: false results should be cited and discussed. Moreover, it would be highlighted that this procedure may be useful in cases with small number of cells (advantage with respect to molecular exams?]

**Treatment**

*Primary Vitreoretinal lymphoma*

From a clinical standpoint, a variable proportion of patients with PVRL experience CNS dissemination, whereas disease remains confined to the eyes for months-years in others. The goals of the treatment of PVRL are to control intraocular disease and to prevent CNS dissemination. The level of evidence supporting therapeutic decisions in PVRL is very low because related literature is sparse and fragmentary, and the main open question regards the distinction of patients who can be managed with ocular treatment alone, and those patients who need systemic chemotherapy. Accordingly, some investigators have reviewed multicenter retrospective series of patients with primary or secondary intraocular lymphoma with the aim to distinguish parameters predicting CNS dissemination ([Grimm*, et al* 2008](#_ENREF_36), [Grimm*, et al* 2007](#_ENREF_37), [Riemens*, et al* 2015](#_ENREF_60)). Unfortunately, reported studies display several selection and interpretation biases, and predicting parameters remain to be defined. Major criticisms of reported studies are the lacking of central pathology review, which is a relevant drawback as the modest diagnostic efficacy of vitrectomy, and the confounding factor determined by the specialty of investigators performing case collection ([Ferreri 2015](#_ENREF_29)). In fact, the 3-year CNS relapse rate is 60% at in the series collected by neuro-oncologists ([Grimm*, et al* 2007](#_ENREF_37)), and 36% in the series collected by ophthalmologists ([Riemens*, et al* 2015](#_ENREF_60)). Other limitations of reported studies regard the inclusion of small patient subgroups receiving variegated treatments, analyzed together in an arbitrary way, and spanning a long period, during which diagnosis and treatment of CNS lymphomas changed greatly.

Albeit with the above-mentioned biases, large retrospective studies seem to suggest that patients with newly diagnosed PVRL should be treated with local strategies, keeping the so-called “extensive treatments” for brain relapses ([Grimm*, et al* 2007](#_ENREF_37), [Riemens*, et al* 2015](#_ENREF_60)). This seems to be a suitable approach because it is associated with negligible systemic toxicity and, conversely to intravenous high-dose methotrexate, intravitreal chemotherapy results in prolonged therapeutic drug concentrations and ocular irradiation allows a better local control ([Ferreri*, et al* 2002](#_ENREF_30)). In fact, patients receiving ocular treatment alone show a good local disease control, with only 13% intraocular relapse rate at a median follow-up of 4 years ([Riemens*, et al* 2015](#_ENREF_60)). However, the major concern when managing PVRL with local treatment alone remains the risk of CNS dissemination, which is a devastating event, occurring in half of patients ([Grimm*, et al* 2007](#_ENREF_37)), and resulting in a 4-year overall survival (OS) of 32%. Conversely, patients with PVRL receiving systemic chemotherapy had a CNS relapse rate of 43%, but the reported 4-year OS is 85% ([Riemens*, et al* 2015](#_ENREF_60)). These data seem to favor “extensive treatments” over “ocular treatments”. The combination of both treatments has not been adequately assessed since ocular therapies were often combined with suboptimal systemic treatments, with consequent disappointing outcome ([Riemens*, et al* 2015](#_ENREF_60)).

In summary, available studies suggest that some patients with PVRL can be safely treated with local treatment alone, while other patients should be treated with systemic chemotherapy. Unfortunately, efforts to distinguish the best candidates for each strategy remain unfruitful owing to relevant selection and interpretation biases, and international efforts aimed to distinguish predictors of CNS dissemination are needed ([Ferreri 2015](#_ENREF_29)). In the meantime, both high-dose methotrexate-based chemotherapy (with or without WBRT) and ocular therapy (intravitreal chemotherapy or ocular radiotherapy) are acceptable strategies ([Hoang-Xuan*, et al* 2015](#_ENREF_39)).

*PCNSL with ocular involvement*

There are no reasons to treat PCNSL patients with ocular disease differently than the rest of PCNSL patients ([Hoang-Xuan*, et al* 2015](#_ENREF_39)). Typically, patients with PCNSL are treated with high-dose methotrexate (MTX)-based polychemotherapy followed by consolidative whole-brain irradiation or autologous stem cell transplantation. However, the eye is a chemotherapy sanctuary where PCNSL tumor cells can grow undisturbed. Chemotherapy efficacy depends on intraocular pharmacokinetics, which are not well understood for most cytostatics. Systemic administration of MTX and cytarabine can yield therapeutic drug levels in the intraocular fluids and clinical responses have been documented; however, drug concentrations in vitreous humor are unpredictable and intraocular relapse is common ([Batchelor*, et al* 2003](#_ENREF_4)). Although the inclusion of both eyes in the irradiation volume seems to improve disease control ([Ferreri*, et al* 2002](#_ENREF_30), [Hoang-Xuan*, et al* 2015](#_ENREF_39)), many researchers are concerned by the risk of lymphoma cells persistence into the eyes, with the consequent increased risk of tumor relapse. Consequently, a benefit from the addition of direct intravitreal injection of cytostatics has been hypothesized, and a large retrospective study has suggested that this strategy is associated with improved PFS ([Grimm*, et al* 2008](#_ENREF_36)), but its effect on OS remains to be defined. Intravitreal MTX is highly effective but does not affect OS, and is associated with important side effects in 73% of eyes and significant deterioration of visual acuity in 27% of patients ([Frenkel*, et al* 2008](#_ENREF_33), [Smith*, et al* 2002](#_ENREF_67)).

Another agent administered in patients with PVRL, with and without any evidence of concomitant cerebral disease, includes intravitreal rituximab, anti-CD20 monoclonal antibody, either alone or in combination chemotherapy with intravitreal MTX. Most data regarding the efficacy of rituximab in treating PVRL currently comes from case reports and a few retrospective case series ([Hashida*, et al* 2012](#_ENREF_38), [Itty and Pulido 2009](#_ENREF_40), [Turaka*, et al* 2012](#_ENREF_70), [Vosganian*, et al* 2011](#_ENREF_72)). Although these authors suggest that rituximab is safe and efficacious in PVRL, these results do further emphasize the need for prospective clinical trials in competitive treatments for this aggressive disease.

**Prognosis and prognostic markers of PVRL**

To date, there are very little data around concerning pathological biomarkers, predicting the prognosis of PVRL. For PCNSL, the International Extranodal Lymphoma Study Group (IELSG) has identified five clinical variables that correlate with prognosis, three are shared with systemic NHL: elevated liver dehydrogenase (LDH), age greater than 60, and the Eastern Cooperative Group performance status greater than 1; parameters specific to PCNSL include elevated CSF protein as well as tumor location within the deep regions of the brain (periventricular, basal ganglia, brainstem and/or cerebellum). The presence of 0-1, 2-3, or 4-5 adverse risk factors correlates with 2-year survival rates of 80%, 48% or 15% ([Ferreri*, et al* 2003](#_ENREF_31)) While the IELSG considered age 60 years to be the cut-point above which prognosis declines, the Memorial Sloan-Kettering prognostic index employs a cut-off point of age 50 ([Abrey*, et al* 2006](#_ENREF_1)). These prognostic models can only be used in PVRL if there is concomitant cerebral disease. A clinical prognostic index for PVRL alone has yet not been devised, but could be an output of the above-mentioned international PVRL registry.

**Conclusion**

Though no standardized recommendations exist for the diagnosis and treatment of PVRL, significant advances have been made in the field in the last decades, in the earlier detection of the disease both clinically and using novel pathological tests, as well as in the efficacy testing of combined radiotherapy and chemotherapy. The advent of biologics such as rituximab does create hope in the community for a targeted and efficacious therapy. Multicenter collaborative international registries and carefully designed clinical trials with associated translational research are the only way forward to better understand the pathogenesis of PVRL, to determine the true relative efficacy and tolerability of available therapies and to identify abnormal pathways that could lead to the use of new target agents.

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**Table 1: Summary of the morphological, immunophenotypical, genotypical and clinical features known to date for the various intraocular lymphoma subtypes.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Intraocular Lymphoma** | **Vitreoretinal** | **Uveal**  *Primary* | **Uveal**  *Secondary* |
| **Most**  **Common**  **WHO Subtype** | DLBCL | EMZL | Dependent on systemic NHL (majority DLBCL) |
| **Immunoprofile** | CD79a+  CD20+  PAX5+  BCL2+  BCL6+/-  MUM1/IRF4+  CD10-/+  CD43-  IgM+  Ki-67 rate: high (>80%) | CD79a+  CD20+  PAX5+/-  BCL2 +  BCL6-  MUM1/IRF4+/-  CD10-  CD43+/-  IgM+  Ki-67 rate: low  (5-15%) | Dependent on systemic NHL |
| **Genotype** | High somatic IgH mutation load  Few ongoing somatic mutations  Chromosomal translocations: t(14;18)(q31;q21)  *MYD88* mutations in ~70% | Low-to-moderate somatic IgH mutation load  Few ongoing somatic mutations  Chromosomal abnormalities:  t(11;18)(q21;q21)  *MYD88* mutations – not known | Dependent on systemic NHL |
| **Putative Cell of Origin** | Possibly 2 different types:  a) Early post-germinal centre B cell = DLBCL of ABC type (majority)  b) Germinal centre cell = DLBCL of GCB type  (minority) | Post-germinal centre (memory) B cell | Dependent on systemic NHL |
| **Clinical Features** | Typically present in 6th and 7th decade  Symptoms:  “Floaters”  Painless decrease  in visual acuity  Signs:  Vitreous infiltrates, possibly with  subretinal involvement.  Rare involvement of choroid  Often bilateral  RPE changes on  FA with ‘leopard skin’-like alterations caused by RPE hyper- and hypopigmentation.  CNS involvement  (70–80% of pts)  Treatment: chemotherapy+/-radiotherapy+/-  Poor prognosis | Typically present in 4th and 5th decade  Symptoms:  Blurring of vision  Metamorphopsia  Signs:  Clear vitreous  Diffuse  thickening of iris and/or choroid  Usually unilateral  Extraocular  extension  frequent  No CNS  involvement  Treatment: low dose radiotherapy.  Good prognosis | Typically >60 yrs  Symptoms:  Previous history of systemic NHL  Decrease in VA  Signs:  Clear vitreous  Diffuse  thickening of iris or and/or choroid  Uni- or bilateral involvement  Concurrent secondary CNS involvement possible  Treatment: dependent on NHL subtype  Poor prognosis |

**Key:** DLBCL = diffuse large B-cell lymphoma; EMZL = extranodal marginal zone B-cell lymphoma; NHL = Non Hodgkin’s lymphoma; CD = cluster of differentiation; ABC = activated B-cell type; GCB = germinal centre B-cell type; VA = visual acuity; RPE = retinal pigment epithelium; FA = fluorescein angiography.