Molecular diagnosis of primary CNS lymphoma in 2024 using *MYD88*^{Leu265Pro} and IL-10

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Early diagnosis is crucial for the successful treatment of primary CNS lymphoma (PCNSL), a rapidly progressing tumour. Suspicion raised on brain MRI must be confirmed by a histopathological diagnosis of a tumour specimen collected by stereotactic biopsy. In rare cases, cerebrospinal fluid (CSF) or vitreous humour might aid in providing a cytological diagnosis. Several disease-related, patient-related, and treatment-related factors affect the timing and accuracy of diagnosis and patient outcome. Some molecules detected in CSF, aqueous and vitreous humour, and peripheral blood were proposed as diagnostic biomarkers for PCNSL; however, detection methods for most of these molecules are not yet standardised, have a long turnaround time, are expensive, and have little reproducibility among labs. By contrast, the *MYD88*^{Leu265Pro} somatic hotspot mutation, revealed by PCR-based assay, is currently and reliably used during the diagnosis of some lymphomas, and IL-10, measured by enzyme-linked immunosorbent assay, is routinely used to diagnose and monitor different common metabolic and immunological diseases. Several independent studies have shown that *MYD88*^{Leu265Pro} and IL-10 can be easily assessed in peripheral blood, plasma, aqueous and vitreous humour, and CSF of patients with PCNSL with substantial sensitivity and specificity, especially when evaluated in combination. In this Viewpoint, evidence supporting the routine use of *MYD88*^{Leu265Pro} and IL-10 in diagnosing PCNSL is considered, and some examples of the frequent difficulties found in the diagnosis of PCNSL are provided, highlighting the role and indications of these two biomarkers to improve the timely recognition of this aggressive tumour.

Early diagnosis for primary CNS lymphoma

Early diagnosis is key for patients with primary CNS lymphoma (PCNSL).[1,2] Given the aggressiveness and the location of disease, a higher chance of complete neurological recovery and a lower risk of developing potentially fatal complications are related to the establishment of prompt and appropriate treatment compared with delayed treatment;[3–5] however, limitations in current diagnostic techniques and the low sensitivity of clinical indicators make early recognition of PCNSL an important clinical challenge.

PCNSL is a diffuse large B-cell lymphoma that exclusively affects the brain, leptomeninges, cranial nerves, eyes, or spinal cord, or a combination of these areas. This lymphoma is classified as a large B-cell lymphoma of immune-privileged sites in the 2022 Edition of the WHO Classification of Haematolymphoid Tumors[6] and is considered a specific entity by the International Consensus Classification of Mature Lymphoid Neoplasms.[7] Symptoms are often non-specific and vary according to disease location. Suspicion is usually based on brain MRI.[8] Differential diagnosis with neoplastic, inflammatory, or infectious diseases can be difficult due to poor and inconclusive data.[9–12] Sometimes modern neuroimaging fails to differentiate PCNSL from high-grade gliomas,[13] which complicates surgical planning as gross tumour resection is the primary curative treatment for high-grade gliomas but is not usually advised for PCNSL.[14] Histopathological assessment of tumour tissue collected by stereotactic brain biopsy is the gold standard for PCNSL diagnosis, reducing the risk of unnecessary and irreversible sequelae related to more extensive surgical approaches.[15,16] This strategy has a high success rate, but obtaining an adequate tumour specimen from deep areas of the CNS, or in unfit or frail patients, can result sometimes in unacceptably high risk of morbidity or mortality. In some patients, lymphoma diagnosis can be achieved by conventional cytology or flow cytometry analysis of cerebrospinal fluid (CSF) or vitreous humour. The assessment of clonality of lymphoid populations by detection of clonal immunoglobulin rearrangements is a well established method recommended to improve diagnostic sensitivity in difficult cases; however, the small number of tumour cells in these CNS compartments often hampers definitive diagnosis.[17] Technical and procedural limitations often lead to diagnostic delays, negatively affecting outcomes.[18] Prolonged corticosteroid exposure before biopsy, often prescribed to manage symptoms, is another source of diagnostic delay because it causes tumour regression (the so-called vanishing tumour), resulting in misleading findings for radiologists and pathologists.[19,20] Equally important, the improper use of corticosteroids carries a high risk of metabolic and infectious complications during anti-PCNSL treatment.

Primary vitreoretinal lymphoma (PVRL) is a form of PCNSL that only shows clinical evidence of disease in the eyes. Diagnosing PVRL can be challenging as it often resembles corticosteroid-resistant chronic uveitis with associated vitritis and has a subtle onset. PVRL belongs to the category of rare diseases known as uveitis masquerade syndromes. These diseases can be either neoplastic or non-neoplastic and present similar symptoms to various infectious and autoimmune conditions.[21]

The diagnostic sensitivity and specificity of the currently recommended approaches must be improved in order to address insidious clinical presentations. This improvement represents an important unmet clinical need with relevant effects on PCNSL outcomes (panel).

The promise of molecular diagnostics for PCNSL

Liquid biopsy has been introduced into the experimental diagnostic investigation of patients with PCNSL with the aim of supporting diagnoses, monitoring responses, and predicting relapses.[23] Several approaches have been investigated, including the evaluation of circulating tumour DNA mutation analysis or microRNAs and the dosing of cytokines or chemokines such as IL-10, IL-6, and CXCL-13 in blood, CSF, and ocular fluids (vitreous and aqueous humour);[24,25] however, most of these approaches are not routinely used due to non-standardised methods, time and resource consumption, and poor reproducibility and validation. The main exceptions are the *MYD88*^{Leu265Pro} somatic hotspot mutation and IL-10 (table). The *MYD88*^{Leu265Pro} mutation has been widely detected by PCR-based assays with consistent results in terms of specificity and is currently used to diagnose and manage certain lymphomas.[53] IL-10, which is routinely used to diagnose or monitor several common metabolic and immunologic diseases,[54–56] has been measured by assays such as enzyme-linked immunosorbent assay, flow cytometry, and electrochemiluminescence immunoassay with high sensitivity and specificity, although a universal positivity threshold has not been established.

The *MYD88* gene is the most frequently mutated gene in patients with PCNSL and other lymphomas arising in immune-privileged sites.[57,58] The *MYD88*^{Leu265Pro} hotspot mutation has been identified in up to 92% of CSF samples[26–33] and up to 83% of ocular fluid samples[44,46,47,49] collected from patients with PCNSL (table). IL-10, a protein secreted in these lymphomas by neoplastic large B lymphocytes,[29] acts as an autocrine growth factor because its receptor is also expressed in PCNSL neoplastic cells.[29,35,59] High IL-10 concentrations have been detected in both CSF and intraocular fluids[29,34–43,45,48] and have been statistically significantly higher in patients with PCNSL than in non-neoplastic control participants (table).

Studies have shown that *MYD88*^{Leu265Pro} and IL-10, assessed in CSF and aqueous and vitreous humour of patients with PCNSL, have high sensitivity and specificity rates, especially when assessed in combination (table).[29,32] In the PAMINA study, the only prospective trial addressing the diagnostic role of CSF biomarkers in PCNSL patients, the *MYD88* gene was evaluated using a TaqMan-based PCR assay on CSF samples collected from 36 treatment-naive patients with PCNSL, 27 patients with relapsing PCNSL, and a control cohort of 162 participants consisting of 118 patients with other brain diseases and 44 patients with non-CNS diffuse large B cell lymphomas.[29] The sensitivity of *MYD88*^{Leu265Pro} at initial diagnosis was 72% with a specificity of 99%. The concomitant assessment of *MYD88*^{Leu265Pro} and IL-10 concentration in the CSF resulted in a sensitivity of 94% and specificity of 98% in treatment-naive patients (area under the curve 0·96; 95% CI 0·91–1·00). Only four control participants had a positive biomarker (ie, either *MYD88*^{Leu265Pro} or high IL-10 concentration). Importantly, none of the 103 assessable controls simultaneously showed both *MYD88*^{Leu265Pro} and high IL-10 concentrations, reinforcing the concept that both positive markers are exclusively associated with PCNSL. The simultaneous detection of IL-10 and IL-6 has also been investigated showing high sensitivity and specificity when assessed in ocular fluids, and variable results have been reported in CSF with sensitivities ranging from 66% to 95% (table). In the PAMINA study, high CSF IL-6 concentrations (>2·5 pg/mL) were found in 24 (73%) of 33 patients with newly diagnosed PCNSL, but also in 38 (48%) of 79 neurological controls and in six (14%) of 44 patients with diffuse large B cell lymphoma, suggesting a low discriminatory sensitivity and specificity of this cytokine in this compartment.

On these grounds, current evidence suggests that *MYD88*^{Leu265Pro} and IL-10 are biomarkers that are potentially useful for early PCNSL suspicion in patients

Panel: **Potential benefits of the routine incorporation of molecular markers into the diagnostic investigations of patients with suspected primary CNS lymphoma**

Diagnostic benefits

- Avoidance of superfluous tumour resection (high-grade gliomas *vs* primary CNS lymphoma)[14]
- Avoidance of improper use of response to corticosteroids as a dignostic surrogate
- Avoidance of high-dose and prolonged use of corticosteroids causing so-called vanishing tumours[19]
- Fewer morphological changes (histopathological puzzle)[22]
- Less neuroradiological misleading (confusing images appearance)

Clinical benefits

- Prompt initiation of specific treatment
- Reduced risk of development of corticosteroid-related complications (metabolic and infectious)
- Higher chances of symptom improvement or resolution (faster neurological recovery)
- Organ function preservation (ocular involvement and cognitive functions)
- Better outcomes (progression free survival and overall survival)
- Improved quality of life and performance status (critical prognostic factor)

Socioeconomic benefits

- Reduced number of instrumental and clinical evaluations before diagnosis
- Reduced inpatient or outpatient admission for treatment related complications
- Reduced need for caregivers or external support due to chronic sequalae (maintained autonomy)
- Increased likelihood of full recovery to precancer quality of life

	Molecular target (cutoff value)	Patients (n)	Control participants (n)	Samples	Assay	Sensitivity (% [AUC; 95% CI])	Specificity (% [AUC; 95% CI])
Studies addressing *MYD88* mutations in CSF samples							
Hiemcke-Jiwa et al (2019)[26]	*MYD88*^{Leu265Pro}	Retrospective: 29 with PCNSL; prospective: 9 with PCNSL	Retrospective: NA; prospective: 19 neurological, infectious, or tumoural diseases other than PCNSL	Retrospective CSF: 17 before treatment, 12 during treatment; prospective CSF: 11 samples from 9 patients with PCNSL; 20 samples from 19 control participants	ddPCR	Retrospective: 6/17 (35%) before treatment, 3/12 (25%) during treatment; prospective: 8/11 (73%) PCNSL, 0/20 (0%) control	Retrospective NA; prospective 100%
Rimelen et al (2019)[27]	*MYD88*^{Leu265Pro}	14 with PCNSL	10 with non-lymphomatous lesions	PCNSL: 9 CSF samples at initial diagnosis and 5 CSF samples at relapse	ddPCR and allele-specific PCR	12/14 (86%)	100%
Watanabe et al (2019)[28]	*MYD88*^{Leu265Pro} and *MYD88*^{Ser219Cys}	21 with PCNSL and 5 with SCNSL	NA	PCNSL: 14 CSF samples at initial diagnosis and 7 CSF samples at relapse	ddPCR and sanger	20/26 (77%)	NA
Ferreri et al (2021)*[29]	*MYD88*^{Leu265Pro} IL-10 (>2 pg/mL)	36 with PCNSL	162 control participants: 118 with CNS disorders and 44 with extra-CNS lymphomas	CSF	TaqMan PCR ELISA	PCNSL: *MYD88* 26/36 (72%), IL-10 32/36 (88%); *MYD88* and IL-10 34/36 (94%); control: *MYD88* 2/162 (1%), IL-10 2/162 (1%)	*MYD88* 99%; IL-10 99% (AUC 0·94; 95% CI 0·86–1·00); *MYD88* and IL-10 98% (AUC 0·96; 95% CI 0·91–1·00)
Gupta et al (2021)†[30]	*MYD88*	52 with PCNSL	65 miscellaneous glioma, Waldenström and other haematological malignancy, metastasis, inflammatory, or others (thyroid carcinoma, melanoma, and inconclusive diagnosis)	CSF	TetRS	66% (95% CI 56·2–74·5)	100% (95% CI 83·9–100·0)
Yamagishi et al (2021)[31]	*MYD88*^{Leu265Pro}	39 with PCNSL and 3 with SCNSL	1 non-CNS lymphoma‡	CSF	ddPCR	92%	100%
Bravetti et al (2023)§[32]	*MYD88*^{Leu265Pro}; cytokines: IL-10 and IL-6	54 with PCNSL	NA	PCNSL: 41 CSF samples at initial diagnosis and 13 CSF samples at relapse	*MYD88*^{Leu265Pro}: RFLP and ddPCR; IL-10 and IL-6: flow cytometry	*MYD88* 28/54 (52%); cytokines 36/54 (67%)	NA
Yamaguchi et al (2023)[33]	*MYD88*^{Leu265Pro}	10 with suspected PCNSL	14 suspected malignant brain tumours	Intraoperative tumour tissue and CSF	RT-PCR¶	Intraoperative tumour tissue 8/10 (80%); CSF 2/2 (100%)	100%
Studies addressing IL-10 concentrations in CSF samples							
Sasayama et al (2012)‖[34]	IL-10 (9·5 pg/mL), detection limit 2 pg/mL	24 with PCNSL	2 primary CNS T-cell non-Hodgkin lymphoma tumours and 40 other brain tumours	CSF	ELISA	71%	100%
Rubenstein et al (2013)**[35]	IL-10 (>16·15 pg/mL)	65 with PCNSL and 23 with SCNSL	137	CSF	PCNSL: RT-PCR; SCNSL: ELISA	64% (AUC 0.851; 95% CI 0·789–0·901)	94% (AUC 0.851; 95% CI 0·789–0·901)
Sasagawa et al (2015)‖[36]	IL-10 (3 pg/mL), median 28 pg/mL	15 with PCNSL and 4 with SCNSL	26 brain tumours and inflammatory diseases	CSF	ELISA	95%	100%
Nguyen-Them et al (2016)††[37]	IL-10 (4 pg/mL), detection limit 2·5 pg/mL	112 (103 PCNSL with or without vitreoretinal lymphoma and 9 PVRL)	40 primary or secondary brain tumours or inflammatory, infectious, or neurodegenerative diseases	CSF	Flow cytometry	87%	89%

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	Molecular target (cutoff value)	Patients (n)	Control participants (n)	Samples	Assay	Sensitivity (% [AUC; 95% CI])	Specificity (% [AUC; 95% CI])
(Continued from previous page)							
Song et al (2016)‡‡‡[38]	IL-10 (8·2 pg/mL); IL-10 to IL-6 ratio (0·72)	22 (19 PCNSL with or without vitreoretinal lymphoma and 3 PVRL)	80 miscellaneous diseases other than CNS lymphoma	CSF	ECLIA	IL-10 96% (AUC 0·957; 95% CI 0·901–1·000); IL-10 to IL-6 ratio 95% (AUC 0·976; 95% CI 0·929–1·000)	IL-10 96% (AUC 0·957; 95% CI 0·901–1·000); IL-10 to IL-6 ratio 100% (AUC 0·976; 95% CI 0·929–1·000)
Mabray et al (2016)§§[39]	IL-10 (20·6 pg/mL)	38 PCNSL	53 primary brain tumours or metastases	CSF	ELISA	95%	95%
Shao et al (2020)¶¶[40]	IL-10 (8·3 pg/mL), median 56 pg/mL; IL-10 to IL-6 ratio (1·6), median 25	66 with PCNSL	42 other types of brain tumours	CSF	Bio-plex multiplex assay	IL-10 59%; IL-10 to IL-6 ratio 66%	IL-10 91%; IL-10 to IL-6 ratio 98%
Ungureanu et al (2021) ‖‖[41]	IL-10 to IL-6 ratio (1·0), median 3·89	28 with PCNSL	15 CNS inflammatory diseases with pseudotumoural lesions	CSF	Flow cytometry	89%	100%
Geng et al (2021)[42]	IL-10 (10·13 pg/mL)	38 with PCNSL	53 other brain tumours	CSF	ECLIA	97%	100%
Studies addressing *MYD88* and IL-10 in ocular humour							
Cassoux et al (2007)[43]	IL-10 (aqueous humour 50 pg/mL and vitreous humour 400 pg/mL)	51 with primary intraocular lymphoma	108 uveitis	Aqueous humour and vitreous humour	ELISA	Aqueous humour 89%; vitreous humour 80%	93% aqueous humour; 99% vitreous humour
Bonzheim et al (2015)[44]	*MYD88*^{Leu265Pro}	21 with confirmed vitreoretinal lymphoma	48 suspected but not confirmed vitreoretinal lymphoma	Vitreous humour (75 eyes)	PCR and DNA sequencing	91%	96%
Costopoulos et al (2016)[45]	IL-10 and IL-6, detection limit 2·5 pg/mL	86 intraocular lymphoma (training cohort) and 25 intraocular lymphoma (validation cohort)	312 non-intraocular lymphoma (training cohort) and 87 non intraocular lymphoma (validation cohort)	Aqueous humour and vitreous humour	Flow cytometry	93%	95%
Hiemcke-Jiwa et al (2018)***[46]	*MYD88*^{Leu265Pro}	23 with PVRL	40 with uveitis	PVRL: vitreous humour (21 eyes) and aqueous humour (27 eyes); control: vitreous humour (20 eyes) and aqueous humour (28 eyes)	ddPCR	ddPCR PVRL 75% (95% CI 50–92); control 67% (95% CI 42–92)	100%
Miserocchi et al (2019)†††[47]	*MYD88*^{Leu265Pro}	8 with PVRL	8 with non-infectious uveitis	PVRL: vitreous humour (8 eyes) and aqueous humour (15 eyes)	TaqMan PCR	PVRL vitreous humour 6/6 (100%); PVRL aqueous humour 6/8 (75%); control 0/8 (0% aqueous humour)	100%
Kuo et al (2020)‡‡‡[48]	ISOLD score; IL-10 to IL-6 ratio	77 with PVRL	84 with uveitis	PVRL: 10 aqueous humour and 67 vitreous humour; control: 19 aqueous humour and 65 vitreous humour	ELISA	ELISA ISOLD 93% (95% CI 83·89–97·61); IL-10 to IL-6 ratio 94% (85·82–98·40)	ISOLD 100% (95% CI 94·48–100·00); IL-10 to IL-6 ratio 95% (87·10–99·04)
Demirci et al (2023)§§§[49]	*MYD88*^{Leu265Pro}	14 with biopsy-confirmed or clinically diagnosed vitreoretinal lymphoma	3 with biopsy-confirmed vitritis	Aqueous humour (18 eyes)	Allele-specific PCR	15/18 (83%)	100%

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	Molecular target (cutoff value)	Patients (n)	Control participants (n)	Samples	Assay	Sensitivity (% [AUC; 95% CI])	Specificity (% [AUC; 95% CI])
(Continued from previous page)							
Studies addressing *MYD88* and IL-10 in plasma or serum							
Hiemcke-Jiwa et al (2019)[56]	*MYD88*^{Leu265Pro}	10 with PCNSL	NA	Plasma (25 samples)	ddPCR	10/25 (40%)	NA
Hattori et al (2018)[50]	*MYD88*^{Leu265Pro}	14 with PCNSL	NA	Serum	ddPCR; TDS	ddPCR 8/14 (57%); TDS 0/13 (0%)	NA
Montesinos-Rongen et al (2020)[51]	*MYD88*^{Leu265Pro} CD79B Y196	27 with PCNSL	6 healthy participants and 4 with Waldenstrom disease	Blood	ddPCR	1/27 (4%)	Low
Geng et al (2021)[42]	IL-10 (2·07 pg/mL)	38 with PCNSL	53 with other brain tumours	Serum	ECLIA	59%	83%
Zhong et al (2024)[52] ¶¶¶	*MYD88*^{Leu265Pro}	24 with PCNSL	NA	Plasma	ddPCR	3/24 (13%)	NA

n/N are provided for sensitivity, and AUC and 95% for both sensitivity and specificity, where available. AUC=area under the curve. CBA=cytometric bead array. CSF=cerebrospinal fluid. ddPCR=droplet digital PCR. ECLIA=electrochemiluminescence immunoassay. ELISA=enzyme-linked immunosorbent assay. FFPE=formalin fixed paraffin embedded. HGG=high grade glioma. IGH=immunoglobulin heavy chain. ISOLD=Interleukin Score for Intraocular Lymphoma Diagnosis. NA=not available. PCNSL=primary CNS lymphoma. PTL=primary testicular lymphoma. PVRL=primary vitreoretinal lymphoma. RFLP=restriction fragment length polymorphism. SCNSL=secondary CNS lymphoma. TetRS=termed targeted rapid sequencing. TDS=targeted deep sequencing. *This study evaluated the same biomarkers in 27 patients with relapsed PCNSL with similar results: the combined analysis showed a sensitivity of 88% and a specificity of 98%. *MYD88*^{Leu265Pro} was found in the CSF of one patient with a history of multiple sclerosis and another patient with systemic diffuse large B cell lymphoma who had an extensive infiltration of nasopharynx and clivus, and IL-10 concentrations were elevated in one patient with a histological diagnosis of reactive gliosis and one patient with primary testicular diffuse large B cell lymphoma. †132 prospective specimens (CSF and plasma liquid biopsy), archived specimens, and cell lines representing 117 patients were analysed using a rapid multiplexed genotyping assay combining *MYD88* mutation detection with parallel analysis of telomerase reverse transcriptase promoter, *IDH1*, *IDH2*, *H3F3A*, and *BRAF* point mutations. ‡Blood DNA from a patient without CNS lymphoma was used as the *MYD88*^{WT} control. §*IGH* gene clonality and *MYD88*^{Leu265Pro} were detected on both CSF cell pellets and supernatants. At least one biomarker was detectable in the CSF of 46 (85%) of 54 patients. ¶GeneSoC (Kyorin) is a genotyping system based on real-time PCR using microfluidic thermal cycling technology. ||Other evaluated markers: IL-6, β2 microglobulin, and soluble IL-2 receptor. **Other evaluated marker: CXCL13. CSF biomarker test based on CXCL13 and IL-10 was significantly more sensitive than CSF cytology ($p<0·001$) and flow cytometry ($p<0·05$) in the detection of PCNSL. ††Detectable IL-10 in the CSF at the end of treatment has a negative effect on the progression-free survival of patients in complete remission or partial remission. ‡‡Other markers: IL-6, IL-8, and tumour necrosis factor α. §§This study evaluated the combined use of MRI apparent diffusion coefficient, CXCL13, and IL-10 in diagnosing PCNSL. CXCL-13 with a cutoff value of 262·8 pg/mL showed particularly similar sensitivity and specificity to IL-10. ¶¶CSF concentrations of T-helper 17 cell-related cytokines were evaluated. The best combination was IL-10 and IL-17A, which had a sensitivity of 70% and specificity of 96%. |||IL-10 median concentration was 36 pg/mL. ***A concordance rate of 89% was observed in 12 paired vitreous fluid–aqueous humour samples. †††Results of *MYD88*^{Leu265Pro} mutation in aqueous and vitreous sample were consistent in 7 of the 8 eyes with available samples. ‡‡‡Patients were classified on the basis of the ISOLD. §§§The mutation was less readily detectable in cellular DNA (10 [56%] of 18) compared with cell-free DNA (15 [83%] of 18). ¶¶¶The *MYD88*^{Leu265Pro} mutation was detected in 9 (64%) of the 14 PCNSL tissue samples evaluated.

Table: ***MYD88* and IL-10 in PCNSL diagnosis**

with uncertain clinical presentation and neuroimaging, and for diagnosing PCNSL or PVRL when surgical procedures are contraindicated (eg, due to poor patient conditions, severe organ dysfunction, or increased risk of bleeding), when tumour site is not suitable for biopsy (eg, the brain stem), or when diagnostic samples are unsuitable (eg, small biopsy, geographical misses, or vitreous humours without tumour cells). Other conceivable uses for these biomarkers include response definition and disease monitoring to early detect relapse, but these applications require more investigation.

Herein, we discuss some clinical scenarios where the use of *MYD88* and IL-10 could aid in solving crucial diagnostic challenges for patients with PCNSL managed in everyday practice.

Unfeasible brain biopsy: deep locations and frail patients

Given that histopathological evaluation of tumour tissue collected by stereotactic brain biopsy is considered the most reliable method for diagnosing PCNSL,[14,17,60] clinical presentation of patients is not always straightforward, and physicians might face challenges in obtaining a diagnosis. Patients with PCNSL show several characteristics associated with increased risk of complications related to brain biopsies. Importantly, PCNSL is a rapidly progressing tumour often diagnosed in older patients; in fact, the median age of patients diagnosed in the last decade is 68 years (range 18–91 years), with more than 40% of patients being older than 70 years.[61] Advanced age and tumour aggressiveness are two characteristics that often result in poor fitness and biopsy contraindication. Moreover, 60% of PCNSLs arise in deep areas of the brain, such as the periventricular tissues, brain stem, and posterior fossa,[62] which are often associated with a high risk of bleeding and other severe and irreversible sequelae. Expert neurosurgeons often avoid the biopsy of lesions located in these anatomical sites, whereas according to conventional agreement in oncology, the absence of histopathological diagnosis precludes the use of intensive chemotherapy and suggests patients as candidates for palliative therapy. The substantial high sensitivity and specificity of combined *MYD88*^{Leu265Pro} and high IL-10 concentrations detected in CSF (table), along with the absence of both positive markers in neurological disorders other than PCNSL, led us to propose these biomarkers as surrogate diagnostic tools for PCNSL in patients who are unable to undergo brain biopsy. The usefulness of these biomarkers in this setting is validated by differences in the management and outcome of two clinical cases reported in figure 1 and the appendix (p 2). Clinical course, site of disease, and neuroradiological findings strongly suggested PCNSL in these patients, but brain stem involvement (mesencephalon and pons) in both patients led expert neurosurgeons to avoid biopsy. The patient in figure 1 was diagnosed in 2015, when studies showing a 98–100% specificity of *MYD88*^{Leu265Pro} in the CSF of patients with PCNSL were not yet available

(table). Accordingly, *MYD88*^{Leu265Pro} was used only as a parameter to support PCNSL suspicion and, in the absence of a histopathological diagnosis, the patient was referred to palliative stereotactic irradiation, had early relapse in non-irradiated areas of the brain, had a confirmatory biopsy, received delayed treatment (7 months after onset of symptoms), and died of lymphoma.

Conversely, more recently, the well documented high sensitivity and specificity of the detection of *MYD88*^{Leu265Pro} and high IL-10 concentration were used to establish a surrogate diagnosis of PCNSL in the other patient (appendix p 2). This diagnosis resulted in the initiation of an effective first-line therapy, with only a 1-month delay and successful treatment completion.

Tumour regression and biomarkers: corticosteroid effects

Prescribing corticosteroids to manage neurological symptoms in patients with brain tumours is common practice. PCNSL has a high sensitivity to corticosteroids, with approximately 40% of patients having transient complete response (the so-called vanishing tumour).[19,63] This effect is not exclusively seen in PCNSL and, therefore, cannot be considered a surrogate diagnostic marker.[64] Corticosteroids can cause substantial diagnostic

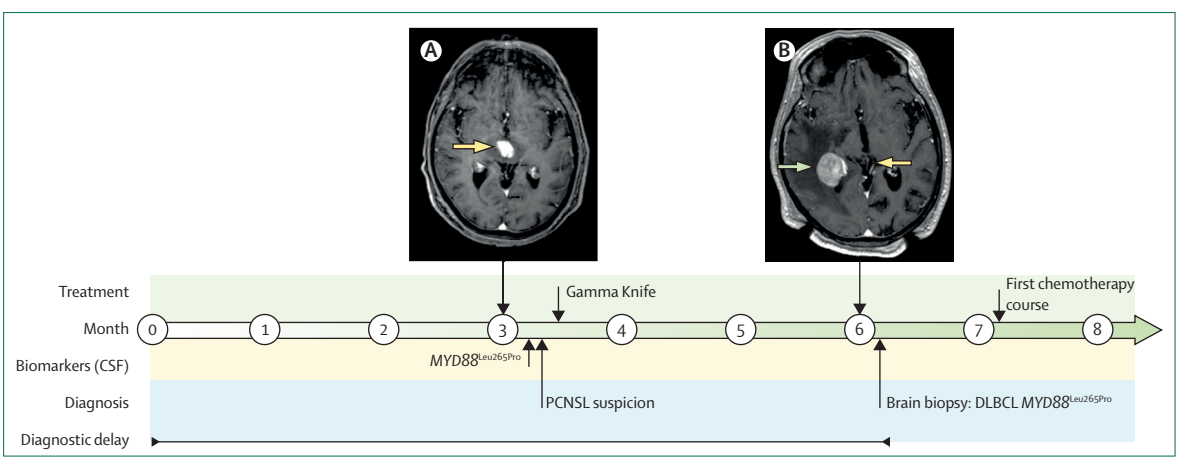

Figure 1: **Clinical case 1**
An older patient with acute dizziness, nausea, and vomiting was diagnosed with frontal ischaemia and vestibular neuronitis. (A) Subsequent brain MRI raised suspicion of lymphoma; the arrow points to contrast-enhanced lesion at the third ventricle on T1-weighted brain MRI). *MYD88*^{Leu265Pro} detection in the CSF led to a provisional diagnosis of PCNSL, and stereotactic radiotherapy was administered. (B) Post-treatment brain MRI showed complete remission of the mesencephalic lesion (yellow arrow) but revealed a new lesion (green arrow). Stereotactic biopsy (performed 6 months after symptom onset) confirmed a DLBCL-harbouring *MYD88*^{Leu265Pro}. Despite treatment containing high-dose methotrexate, the patient worsened and died in 3 months. CSF=cerebrospinal fluid. DLBCL=diffuse large B cell lymphoma. PCNSL=primary CNS lymphoma.

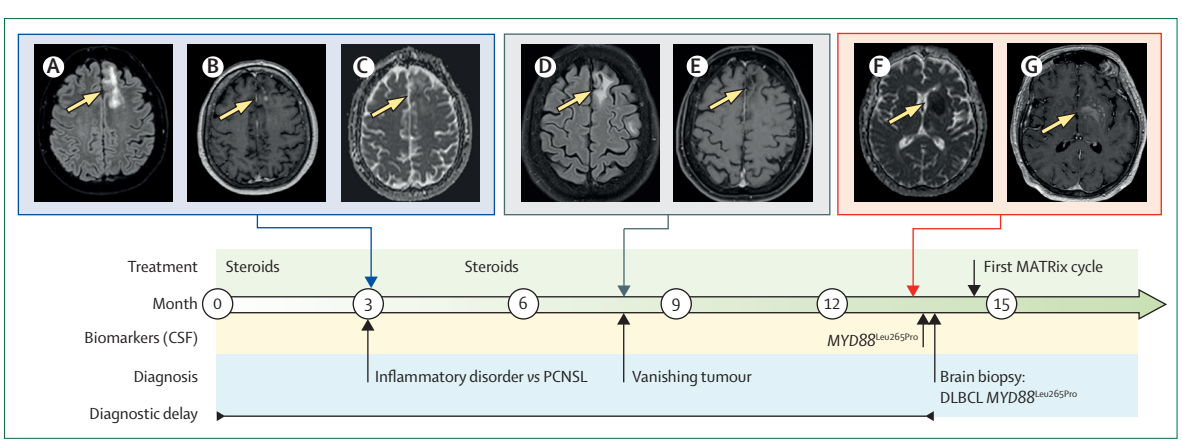

Figure 2: **Clinical case 2**
A healthy individual with personality changes presented a lesion in the left frontal lobe on a brain MRI. Corticosteroids were initiated, revealing an atypical lesion after 2 months with hyperintensity on Flair. The lesion was mainly subcortical (A), showed tiny foci of enhancement in T1 after gadolinium (B) and did not exhibit restriction in diffusion-weighted imaging (C). Prolonged corticosteroid treatment led to a vanishing-tumour effect[19] (regression of the lesion with a residual hyperintensity on Flair [D] and a complete disappearance of enhancement in T1 [E]). A subsequent brain MRI revealed a new lesion in the left basal ganglia, characterised by diffusion restriction (F) and irregular enhancement (G), suggestive of lymphoma.[8] Both *MYD88*^{Leu265Pro} and high IL-10 concentrations in the CSF supported the radiological suspicion of lymphoma,[29] which was confirmed by histopathological examination of biopsied material. MATRix therapy was started, but the patient died shortly after the first cycle due to an infectious complication.[5,65] CSF=cerebrospinal fluid. DLBCL=diffuse large B-cell lymphoma. Flair=fluid attenuated inversion recovery. MATRix=high-dose methotrexate, high-dose cytarabine, thiotepa, and rituximab. PCNSL=primary CNS lymphoma.

delays and negative outcomes. Infectious complications arise in 11–28% of patients per cycle of intensified chemotherapy induction, particularly during the early cycles when the effect of corticosteroids on frail health status and immunosuppression is more relevant.[5] These percentages are clearly illustrated by the patient in figure 2, who had a substantial treatment delay due to a prolonged exposure to corticosteroids that resulted in an alternation of tumour regressions and recurrences for more than 1 year. Once the biopsy was performed, effective treatment was immediately indicated but the patient died due to infectious complications after the first chemotherapy course. The 1-year treatment delay and unfavourable outcome could have potentially been avoided upon the detection of *MYD88* mutation and IL-10 in the CSF.

Another patient, described in the appendix (p 3) shows two potential limitations of biomarkers assessment in CSF. First, contraindications to CSF sampling by lumbar puncture, usually due to progressive intracranial hypertension, impedes the assessment of *MYD88*^{Leu265Pro} and IL-10; thus, these biomarkers should be investigated early during diagnostic investigations when biopsy is not feasible. Second, the confounding effects of corticosteroid use might not be limited to traditional diagnostic techniques (ie, neuroimaging and histological exam), but could also affect interpretation of biomarker results. Lymphoma regression under corticosteroid treatment can be associated with the disappearance of *MYD88*^{Leu265Pro} and normalisation of IL-10 concentrations. Corticosteroids might result in an undetectable DNA yield following fluid sample extraction.[66] Additionally, tumour cell death induced by these drugs might lead to a short-lived increase of circulating tumour DNA in CSF that would then be quickly cleared and reduced to undetectable concentrations.[67] Likewise, some studies have documented that PCNSL regression after chemotherapy is followed by a rapid and substantial reduction of IL-10 concentrations in the CSF (table).[34,37] To date, the effects of corticosteroids on the detection of *MYD88*^{Leu265Pro} and IL-10 are speculative and supported by little data. Nevertheless, assessment of these biomarkers in CSF should be avoided during corticosteroid therapy, especially if a tumour response is confirmed. In patients with PCNSL suspicion, we suggest repeating biomarker assessment on CSF samples if initial results are negative due to the potential effect of corticosteroids on DNA yield and IL-10 production.

Biomarkers and the masquerade syndrome

International guidelines[68] recommend testing IL-10, IL-6, and *MYD88*^{Leu265Pro} in ocular fluids because, although not diagnostic, they are reliable indicators of PVRL[45,48] (table). The onset of disease in the eyes is often insidious and characterised by blurred vision or floaters, or both. Given the multitude of potential differential diagnoses, the indolent nature of the disease's initial course, and improper use of corticosteroids, the diagnostic delay for PVRL can extend up to 21 months.[69] PVRL can ultimately impair visual acuity and lead to

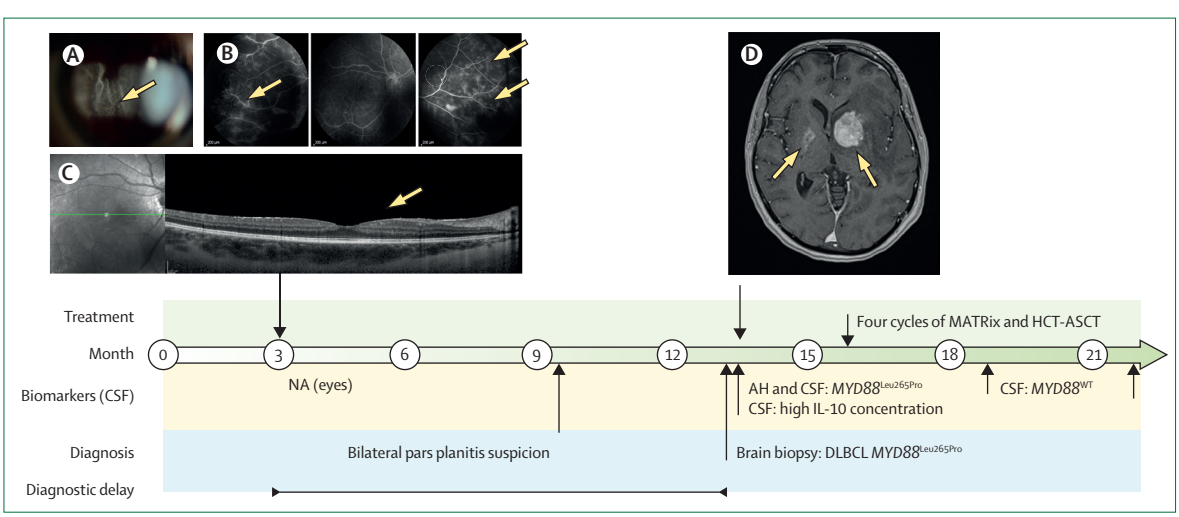

Figure 3: **Clinical case 3**
A young patient with a 3-month history of blurred vision and floaters in both eyes typical of bilateral intermediate uveitis, shown by vitritis at the slit lamp examination (A), peripheral active retinal vasculitis at the fluorescein angiography (B), and normal retinal layers at the optical coherence tomography (C), was diagnosed with bilateral pars planitis after ruling out other common causes.[72] 1 year later, a brain MRI revealed lesions at bilateral basal ganglia suggestive of lymphoma (D). A stereotactic brain biopsy resulted in diagnosis of DLBCL harbouring *MYD88*^{Leu265Pro}. The presence of *MYD88*^{Leu265Pro} in the aqueous tap, which could have been used to diagnose primary vitreoretinal lymphoma 1 year earlier, confirmed the ocular involvement.[47] Complete remission was achieved with 4 cycles of MATRix followed by a carmustine–thiotepa-conditioned autologous stem cell transplantation.[73] Both *MYD88*^{Leu265Pro} and high IL-10 concentration were detected in the CSF[29] before treatment. After the second and fourth MATRix cycles, *MYD88* was shown to be wild-type. AH=aqueous humour. CSF=cerebrospinal fluid. DLBCL=diffuse large B-cell lymphoma. HCT-ASCT=high-dose chemotherapy with autologous stem cell transplantation. MATRix=high-dose methotrexate, high-dose cytarabine, thiotepa, and rituximab. NA=not available.

blindness. Furthermore, within 30 months from diagnosis,[70,71] up to 90% of patients diagnosed with PVRL are prone to developing brain relapse, which is the primary cause of performance status impairment and mortality among these patients. As for other PCNSL, morphology is the standard criterion for the diagnosis of intraocular lymphoma;[68] however, definitive diagnosis of PVRL is only reported in half of suspected cases due to the low number of neoplastic cells and their poor conservative status in vitreous samples. For the patient in figure 3, a thorough investigation was conducted to rule out common causes of vitritis, including infections and autoimmune conditions.[72] This investigation resulted in a diagnostic delay of 1 year, which could have potentially been avoided by early incorporation of the assessment of *MYD88* and IL-10 in ocular humours. Aqueous humour sampling is less invasive than vitreous humour sampling and assessment of *MYD88* and IL-10 produces similar results in both ocular humours.[43,46,47] Accordingly, biomarker assessment on aqueous humour is a reliable procedure to assess intraocular involvement both at diagnosis of PVRL and at staging in patients with PCNSL.

Conclusion

Several independent studies have shown that *MYD88*^{Leu265Pro} and IL-10 concentrations can distinguish PCNSL with high sensitivity and specificity from other neurological (neoplastic and non-neoplastic) disorders. Combined detection of these biomarkers on CSF samples aids in early diagnosis, might act as a surrogate diagnostic approach whenever brain biopsy cannot be performed, and are reliable tools for distinguishing intraocular lymphoma. Conversely, although effective chemoimmunotherapy is followed by a normalisation of *MYD88* and IL-10 in CSF samples in some patients (eg, those in figure 3 and the appendix [p 2]), more investigation is warranted to establish the role of these biomarkers in defining therapeutic response and monitoring disease during follow-up. Herein discussed clinical cases show how *MYD88*^{Leu265Pro} and IL-10 assessed in the CSF or ocular fluids, or both, can assist in obtaining a rapid and safer diagnosis, as well as in driving a multidisciplinary management of patients with PCNSL. Therefore, on the basis of current evidence and our experience, we recommend the routine diagnostic use of these two biomarkers in the presence of complicated clinical scenarios. CSF and ocular fluid samples in their entirety (cell fraction plus its cell-free fraction) should be analysed because of the low percentage of cytologically positive samples and the negligible influence of the cellular compartment on the biomarkers detection rates.[29] The possibility of expanding the search for *MYD88*^{Leu265Pro} in plasma, although of great value due to its easier accessibility, remains very puzzling due to the low detection rates reported in this bodily fluid by other researchers[26,42,50–52] (table) using the same highly sensitive technique (ie, droplet digital PCR).

Search strategy and selection criteria

References for this Viewpoint were identified by searching PubMed for articles published between Jan 1, 2005, and April 15, 2024, using the search terms "biomarkers", "liquid biopsy", "*MYD88*", "interleukin-10", "Cerebrospinal Fluid", "Aqueous Humor", and "Vitreous Humor" in combination with "primary CNS lymphoma". Articles were also identified by searches of the authors' own files and abstracts from recent international haematology and oncology conferences. Only papers and abstracts published in English were reviewed. The final reference list, with few studies published before Jan 1, 2005, was selected based on originality and relevance to the broad scope of this Viewpoint.

In the near future, more sensitive and promising tools will hopefully be implemented and harmonised in the diagnostic, response, and disease-monitoring workflows.[74] Ultrasensitive and integrated multi-omics next-generation sequencing-based technologies have already shown the ability to characterise tumour heterogeneity and improve the sensitivity of tumour DNA detection in the different body fluids. Other potential uses of these targeted gene panels will be to inform treatment and establish prognosis;[75–77] however, these modern techniques are not yet standardised and require full validation in independent and larger cohorts, as well as optimisation of timing and cost, before they are widely used. Looking forward, while we await the standardisation of these ultrasensitive techniques, routine assessment of *MYD88*^{Leu265Pro} and IL-10 measurement could substantially improve the accurate and timely identification of a larger subset of patients with PCNSL.

Contributors

TC and AJMF conceptualised the Viewpoint. TC and MGC visualised the figures. TC and PF performed the review of the literature. NA reviewed and provided brain MRI images. EM performed ophthalmological evaluation and provided related pictures. MGC performed the molecular analyses. FG performed the brain biopsies. MP performed the histopathological analyses. TC wrote the first draft of the manuscript and created the figures. All authors critically reviewed the manuscript and approved the final version.

Declaration of interests

TC received an independent grant (Award for Younger Researchers 2018) from Roche. AJMF was the chairman of the PAMINA trial, which supports the use of *MYD88*^{Leu265Pro} and IL-10 as diagnostic markers in patients with primary CNS lymphoma. All other authors declare no competing interests.

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