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# Molecular analysis in liquid biopsies for diagnostics of primary central nervous system lymphoma: Review of literature and future opportunities

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ABSTRACT

Primary central nervous system lymphoma (PCNSL) is an aggressive lymphoma with a poor prognosis, for which accurate and timely diagnosis is of utmost importance. Unfortunately, diagnosis of PCNSL can be challenging and a brain biopsy (gold standard for diagnosis) is an invasive procedure with the risk of major complications. Thus, there is an urgent need for an alternative strategy to diagnose and monitor these lymphomas.

Currently, liquid biopsies from cerebrospinal fluid (CSF) are used for cytomorphologic and flow cytometric analysis. Recently, new biomarkers such as genetic mutations and interleukins have been identified in these liquid biopsies, further expanding the diagnostic armamentarium.

In this review we present an overview of genetic aberrations (> 70) reported in this unique lymphoma. Of these genes, we have selected those that are reported in ≥3 studies. Half of the selected genes are implicated in the NFκB pathway (*CARD11*, *CD79B*, *MYD88*, *TBL1XR1* and *TNFAIP3*), while the other half are not related to this pathway (*CDKN2A*, *ETV6*, *PIM1*, *PRDM1* and *TOX*). Although this underlines the crucial role of the NFκB pathway in PCNSL, *CD79B* and *MYD88* are at present the only genes mentioned in liquid biopsy analysis.

Finally, a stepwise approach is proposed for minimally invasive liquid biopsy analysis and work-up of PCNSL, incorporating molecular analysis. Prioritization and refinements of this approach can be constructed based upon multidisciplinary collaboration as well as novel scientific insights.

## 1. Introduction

Primary central nervous system lymphoma (PCNSL) occurs within the special sanctuary of the blood-brain barrier and is therefore regarded as an 'immune-privileged (IP)' lymphoma. The majority of PCNSLs are diffuse large B-cell lymphomas (DLBCLs) (Louis et al., 2007; Bhagavathi and Wilson, 2008), which originate from late germinal centre (GC) B-cells (Deckert et al., 2014; Courts et al., 2008; Coupland and Damato, 2008; Alizadeh et al., 2000) or early post-GC B-cells with blocked terminal differentiation (Camilleri-Broet et al., 2006; Braggio et al., 2015; Montesinos-Rongen et al., 2004; Coupland and Damato, 2008). These DLBCLs show many similarities with nodal DLBCLs of the activated B-cell (ABC) type. In fact, PCNSLs are classified as ABC-type in up to 96% of cases (Hattab et al., 2010).

Standard treatment regimen of PCNSL entails high-dose methotrexate based polychemotherapy (Ferreri et al., 2009; Ferreri et al., 2016), but the efficacy of this regimen is variable and disease relapse is common. As a result, overall prognosis is poor with a mean survival period of less than 5 years (Gonzalez-Aguilar et al., 2012; Montesinos-Rongen et al., 2009; Camilleri-Broet et al., 2006), which is significantly worse than in nodal DLBCLs (Gonzalez-Aguilar et al., 2012; Montesinos-Rongen et al., 2009; Camilleri-Broet et al., 2006; Zucca et al., 2003).

Thus, timely diagnosis and early start of treatment are critical to improve prognosis. For PCNSL, histological analysis of biopsy material is still regarded as gold standard. Unfortunately, major complications can arise during the procedure of a brain biopsy, such as intracranial haemorrhage or functional impairment (Khatab et al., 2014). In addition, although biopsy is diagnostic in most cases, sensitivity of these biopsies is decreased in cases where steroids are given before the biopsy

*Abbreviations:* ABC, activated B-cell type; BCM, BCL10, CARD11, MALT complex; BCR, B-cell receptor; BTK, Bruton's tyrosine kinase; CSF, cerebrospinal fluid; GCB, germinal center B-cell type; IL, interleukin; PCNSL, primary central nervous system lymphoma; TLR, toll-like receptor

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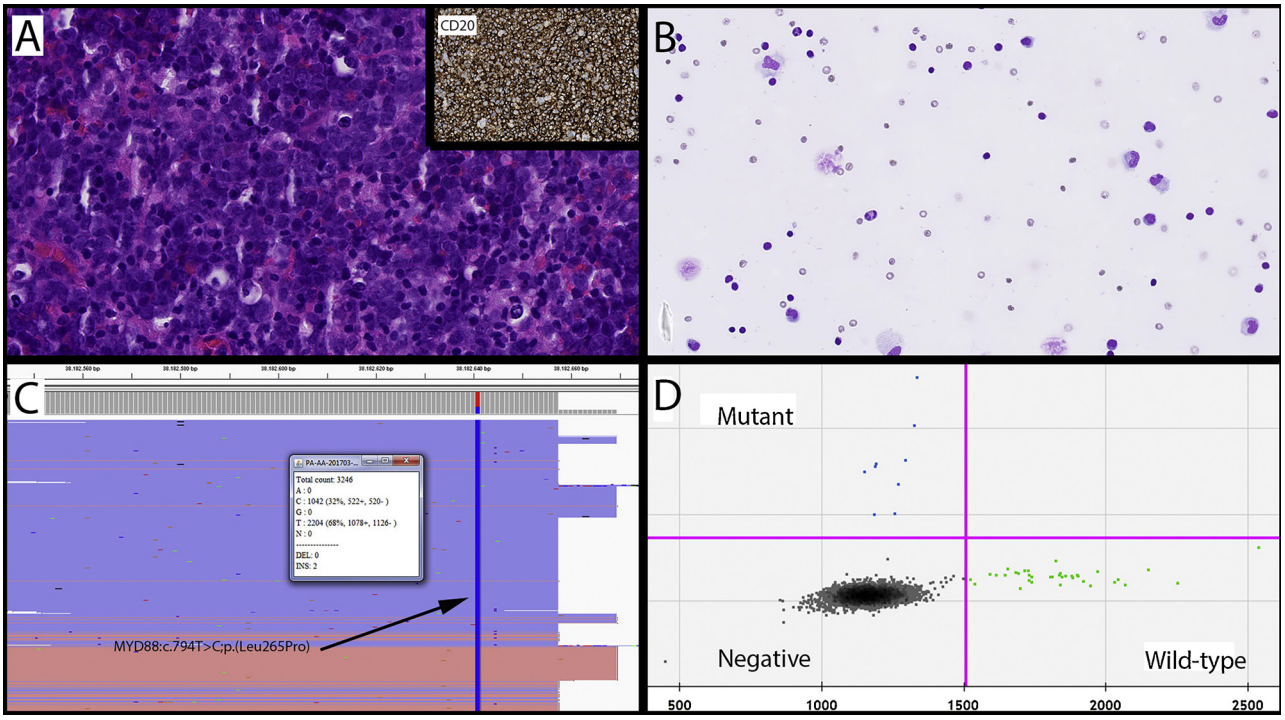


**Fig. 1. Morphology and molecular analysis for *MYD88* of a PCNSL patient.** A. Histomorphology of the brain biopsy showing large, atypical lymphocytes with prominent nucleoli, positive for CD20 (inlet), highly suggestive of diffuse large B-cell lymphoma. B. Cytomorphology showing small lymphocytes without features suggestive of lymphoma. C. Next Generation Sequencing results of the brain biopsy showing presence of the MYD88 p.(L265 P) mutation (arrow: mutation frequency 32%). D. Droplet digital PCR of the cerebrospinal fluid showing presence of MYD88 p.(L265 P) mutant droplets (blue droplets: mutation frequency 17%). Green droplets depict wild-type droplets; gray droplets depict negative droplets without detectable DNA (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

is taken (Onder et al., 2015).

Alternatively, diagnosis of PCNSL can be based upon clinical and radiological characteristics, combined with cytomorphologic and flow cytometric analysis of cerebrospinal fluid (CSF) (Baraniskin and Schroers, 2014). Currently, the advent of lymphoma-specific mutations and unique combinations of cytokines (Sasagawa et al., 2015; Song et al., 2016) that can be tested in CSF has the potential to further increase the diagnostic value of this liquid biopsy for PCNSL.

In this review, we will present an overview of current diagnostic biomarkers for PCNSL and the unmet need for additional, specific biomarkers, especially in liquid biopsy analysis. With this in mind, we will present an overview of established and emerging genetic aberrations mentioned in literature and discuss a selection of these genes with regard to their potential for liquid biopsy analysis as well as their prognostic implications and role in targeted therapy. Finally, we will propose a stepwise approach for liquid biopsy analysis in PCNSL, incorporating molecular analysis.

## 2. Routine biomarkers for diagnostics of PCNSL

### 2.1. Clinical symptoms and imaging

Clinical symptoms of patients with CNS lymphoma, such as cognitive impairment, personality changes, focal neurological deficits and signs of raised intracranial pressure (Schlegel, 2009; Zhang et al., 2010), are similar between PCNSL and secondary CNS lymphoma (SCNSL) and no single symptom, or combination of symptoms, is pathognomonic for PCNSL or SCNSL.

Similarly, radiological findings may suggest the diagnosis of PCNSL or SCNSL, but remain non-diagnostic. This makes it difficult to discriminate PCNSL from SCNSL upon imaging characteristics, as well as from gliomas, demyelinating entities, vasculitis, neurosarcoidosis, infections and cerebral/leptomeningeal dissemination of systemic tumors.

Ocular examination is part of the standard diagnostic work-up of patients suspected with PCNSL. Dilated fundoscopy with slit lamp examination, fluorescent angiography and optical coherent tomography can detect cellular infiltration of the vitreous humor or the subretina (Chan et al., 2011). Unfortunately, these findings are also observed in uveitis, an inflammatory ocular disease, which requires a starkly contrasting treatment approach. In fact, vitreoretinal lymphoma of the eye, either primary or secondary to PCNSL, commonly masquerades as non-infectious uveitis and may initially respond to immunosuppressive therapy.

### 2.2. Tissue biopsies

Although not perfect, histomorphological analysis of tissue biopsies is still regarded as gold standard for diagnosis of PCNSL. Unfortunately, taking these biopsies harbors the risk of major complications for the patient, such as intracranial haemorrhage, functional impairment or loss of vision in case of a chorioretinal biopsy (Khatab et al., 2014; Gupta et al., 2007). In some instances, the tumor is located within or adjacent to critical brain structures, and taking a biopsy is not feasible. Moreover, as steroids are often given before the biopsy is taken to reduce mass effect as a result of edema, histopathological diagnosis can be hampered. This may contribute to the observation of a relatively poor diagnostic sensitivity rate of 48% (Onder et al., 2015), which is inadequately low for a gold standard. Therefore, there is a pressing need for an alternative, less invasive diagnostic strategy.

### 2.3. Current use of liquid biopsies for diagnostics of PCNSL

When PCNSL is suspected on clinicoradiological grounds, a lumbar puncture is often performed for CSF investigation, except in the case of intracranial mass effect with risk of brain herniation. This includes a