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Molecular Profiling of Primary Central Nervous System Lymphomas – Predictive and prognostic value?

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Abstract

Purpose of review: Primary central nervous system lymphoma (PCNSL) is a rare but aggressive variant of non-Hodgkin lymphoma. The diagnostic gold standard remains the pathologic review of tumor tissue, mainly collected though biopsies. The majority of PCNSL are diffuse large B cell lymphoma (DLBCL). Biopsies are invasive procedures, and there have been efforts to develop minimally invasive diagnostic testing using serum and cerebral spinal fluid (CSF). This article reviews multiple markers that potentially could serve as future diagnosis tools and predictors of treatment response.

Recent findings: Many studies have attempted to classify DLBCL into different subtypes for prognostic purposes using methods such as immunohistochemistry. PCNSL often falls under the activated B-cell-like subgroup, and further genomic sequencing has identified genomic alterations in genes within the B-cell receptor signaling axis, e.g. MYD88 or CD79B, at increased frequencies in PCNSL. MYD88 and CD79B implicate the involvement of the NF-kB pathway, and targeted agents to this pathway are currently being used in the treatment of relapsed/refractory PCNSL.

Summary: Although recent genomic profiling of PCNSL has increased the understanding of drivers in this disease and has also led to the introduction of targeted inhibitors, these markers have not yet been used for diagnostic and/or prognostic purposes. Further studies will need to evaluate if they hold great diagnostic potential.

Keywords

PCNSL; B-cell receptor; MYD88; CD79B; activate B-cell subtype

Introduction

Primary central nervous system lymphoma (PCNSL) is a rare but aggressive type of extranodal non-Hodgkin lymphoma found only in the central nervous system. Treatment regimens vary according to geographic location and physician practice, but all include the

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use of high-dose methotrexate. Despite this, the rate of relapse is high, and there are ongoing studies as to which treatment regimen is best for this patient population. The gold standard for diagnosis is the pathologic review of biopsy material. Stereotactic biopsies are an invasive procedure with multiple associated risks such as brain edema, hemorrhage and permanent neurologic deficits. With the advent of genomic sequencing and other technology, efforts have been made to identify additional biomarkers for diagnosis and their prognostic and predictive value. This review aims to identify current work on these potential markers.

Background

PCNSL is found only in the central nervous system, which includes the brain, spine, cerebrospinal fluid (CSF), and eyes [1]. It represents 3% to 4% of all newly diagnosed intracranial neoplasms with an overall incidence rate of 0.5 per 100,000-person years. PCNSL represent 4% to 6% of all extranodal lymphomas. [2].

PCNSL presents as solitary or multiple intracranial lesions, diffuse leptomeningeal or periventricular lesions, vitreous/uveal deposits, and rarely as intradural spinal cord lesions [3-7]. The clinical presentation varies depending on the location of the lesion. Patients may develop mental status and behavioral changes (32% to 43%), seizures (11% to 14%), and signs of elevated intracranial pressure such as headache, nausea, vomiting, and papilledema (32% to 33%) [1, 8, 9].

To diagnose and assess the extent of disease, the International PCNSL Collaborative Group (IPCG) recommends baseline staging to include magnetic resonance imaging of the brain and spine (if symptoms localizing to the spine are present), an ophthalmologic examination to evaluate for intraocular lymphoma, and CSF evaluation to determine the presence of lymphoma in the CSF [10]. A body positron emission tomography/computed tomography scan and bone marrow biopsy should also be performed to detect the presence of non-CNS disease as this would change management strategies. To establish a tissue diagnosis of PCNSL, the diagnostic procedure of choice is a stereotactic biopsy. If ocular or CSF involvement is present, then a vitrectomy or CSF cytology may be collected instead. Molecular markers have not been included in the diagnostic recommendations.

There is currently no standard of care treatment for PCNSL, but expert consensus recommends treating patients with a high-dose methotrexate-based multimodal regimen. Methotrexate was initially added to whole brain radiation therapy, but there was significant long-term neurotoxicity from chemoradiation [11, 12]. This led to the development of chemotherapy-only regimens such as rituximab, methotrexate, vincristine, and procarbazine [13, 14]; rituximab, methotrexate, and temozolomide [15]; methotrexate, cytarabine, thiotepa, and rituximab [16]; and rituximab, methotrexate, carmustine, teniposide, and prednisone [17]. No head-to-head comparisons have been conducted to determine which regimen is the most efficacious; thus, the regimen used often depends on geographic location and physician preference. Ongoing trials will hopefully add to this literature and provide further evidence for the optimal first line treatment. Agents targeting tumor specific alterations have not been introduced into the first-line setting.

Pathology, Pathophysiology and the Tumor Microenvironment

Approximately 90% of PCNSL are diffuse large B cell lymphomas (DLBCL) with a small subset of patients diagnosed with T cell, Burkitt, lymphoblastic, and marginal zone lymphomas [18]. DLBCL are characterized by diffuse proliferation of mature B cells that are usually larger than twice the normal size of macrophages or lymphocytes [19]. It is thought that B-cell lymphomas arise from B cells that arrest at specific stages of differentiation when malignant transformation takes place [20].

Expression profiling data [21] was used to establish three major DLBCL subtypes: 1) germinal center B-cell-like (GCB), 2) activated B-cell-like (ABC), and 3) type 3. The type 3 subgroup is not well defined, but both the type 3 and ABC subtypes appear to have a poor outcome and are often grouped together. For easier clinical application, an immunohistochemical classification scheme for systemic DLBCL was developed by Hans et al subdividing tumors into germinal center B-cell-like (GCB) and non-germinal center like (non-GC) based on the expression pattern for CD10, BCL6, and MUM1 [22].

Further genomic sequencing revealed that the pattern of somatic mutations in DLBCL varies depending on the cell of origin: GCB tumors were more likely to have mutations in *EZH2, GNA13*, and translocations in *BCL2*, whereas ABC tumors were associated with mutations in *MYD88, CD79A, CARD 11,* and *TNFAIP3*, all of which are involved in B-cell receptor signaling activating NF-kB [23].

The majority of PCNSL are of the non-GC subtype [24-26]. In comparison to the ABC subtype in systemic DLBCL, mutations in *MYD88* and *CD79B* are identified in higher frequencies [26-32] (Figure 1) and even found in tumors of the GC subtype in PCNSL, which is not typically observed in systemic DLBCL [26, 33, 34].

The NF-kB pathway plays a key role in DNA transcription and cell survival. Constitutively active NF-kB leads to cell proliferation and prevention of cellular apoptosis and sustains the viability of ABC subtype DLBCL [33, 35]. Bruton tyrosine kinase (BTK) links B-cell receptor (BCR) and toll-like receptor (TLR) signaling pathways to downstream NF-kB activation. In over 90% of PCNSL tissue samples, BCR, TLR, or NF-kB pathways are altered [28]. Fifty-five percent of PCNSL patients have mutations identified within the Toll/IL-1 receptor domain of MYD88, an adaptor protein that activates the exchange of leucine to proline at position 265 leading to the development of DLBCL [36]. In addition, 40% of PCNSL cases have mutations in the immunoreceptor tyrosine-based activation motif of CD79B located at Y196, which leads to chronic activation of BCR signaling and subsequent activation of the NF-kB pathway [37]. This is in contrast to systemic DLBCL cases where MYD88 and CD79B mutations are seen less frequently in ABC subtypes (8-37% MYD88 and 12-22% CD79B) [38-41]. NF-kB activity can also be further amplified through deletions or mutations in tumor necrosis factor alpha induced protein 3 (TNFAIP3) [42].

The *BCL6* gene is a proto-oncogene expressed on normal B-cells in the germinal center that produces the transcriptional repressor protein BCL6, which regulates its own expression by binding to its promotor. BCL6 has many functions, one of which is to represses microRNA expression (ie miR155) leading to increased expression of genes needed for germinal center

reactions to produce antibody diversity [43]. BCL6 also allows for the rapid proliferation of germinal center B-cells in response to T-cell dependent antigens without inducing a p53/TP53-dependent apoptotic response to the physiologic DNA breaks needed for immunoglobulin class switch recombination and somatic hypermutation [44]. Chromosomal translocations of *BCL6* occurring in 30% to 40% of DLBCL or acquired mutations in the *BCL6* promotor occurring in 73% of DLBCL lead to overexpression of BCL6 and subsequent downregulation of the *TP53* tumor suppression gene, resulting in continuous activation and cell proliferation [19, 45]. A subset of ABC DLBCL activates the transcription factor STAT3 (a BCL-6 target) through JAK kinase signaling, which synergizes with NF-kB and promotes cell survival [46, 47]. JAK/STAT signaling pathway activators such as interleukin-4 (IL-4) and IL-10 were found to be upregulated in the PCNSL microvascular environment and in the vitreous and CSF [48].

Given that the CNS is a site that does not have resident lymphoid tissue, it is unknown which cell of origin PCNSL arise from. The brain is immunologically quiet under physiologic conditions, but biopsies from PCNSL patients show an inflammatory response with reactive T cells and infiltrating activated macrophages. In addition, there are highly proliferative tumor cells that diffusely infiltrate the CNS in an angiographic growth pattern migrating in the perivascular space [18, 49]. The tumor microenvironment is not fully characterized, but in vitro studies have found that large B-cell lymphomas respond to chemokines CXCL12 and CXCL13 [50]. The presence of tumor-infiltrating CD14+ macrophages may portend a better treatment response as they provide complement and Fc receptors needed for rituximab to be effective [51]. Macrophages in the PCNSL tumor environment were also found to overexpress programmed death-1 (PD-1), indoleamine 2,3-dioxygenase (IDO1), and several other cytokines in response to in vitro PCNSL cell-line derived soluble factors [52]. Genomic studies of PCNSL samples have identified a frequent 9p24.1/PDL1/PDL-2 copy number gain that is associated with increased PDL1/PDL2 protein. Taken together, this suggests that the expression of immunosuppressive molecules like PD-1 may be involved in immune evasion of lymphoma cells [53].

Another transcription factor that is often upregulated in DLBCL due to translocation is MYC, which drives cell proliferation, regulates cell growth and differential, and apoptosis through downregulation of BCL2 [54, 55]. MicroRNAs associated with the MYC pathway have been identified in PCNSL patients where putative tumor-suppressor microRNAs such as miR-199a, miR-214, miR-193b, and miR-145 were downregulated [56].

Diagnostic Markers

Diagnostic delay is often an issue for PCNSLs given the rapidly progressive nature of the disease, and the gold standard for diagnosis involves a stereotactic biopsy of the lesion [57]. This is invasive and is associated with a complication rate of 8.5% including brain edema, hematomas, or seizures [58]. To minimize this risk, efforts have been made to identify other less invasive methods for diagnosis. Cytology and flow cytometry from the CSF or vitreous fluid are often tested, however the diagnostic yield is low and often only positive when there is significant leptomeningeal or vitreal involvement. Moreover, conventional CSF cytology and flow cytometry testing often does not produce corresponding results. A prospective

study of 123 B-cell lymphoma patients reports that flow cytometry identified neoplastic B cells in 27 patients (22%) while conventional cytology was only positive or suspicious in 10 patients [59].

Additional CSF markers have been assessed. On a systematic review performed by van Westrhenen et al, CXCL-13, B2M, and neopterin isolated from the CSF appear to have the most potential to become a diagnostic marker in PCNSL [60]. Table 1 lists additional CSF markers investigated as potential diagnostic markers for PCNSL [50, 61-77]. To date, none of these markers has been established in the clinical routine diagnostic use.

The identification of circulating tumor DNA (ctDNA) in the CSF and/or blood might be a possibly more promising diagnostic biomarker for PCNSL. Fontanilles et al investigated ctDNA changes in serum and primary tumors in 25 PCNSL. Eight patients (32%) had detectable somatic mutations in the blood. The sensitivity was determined as 24%, with a specificity of 100% [63]. In a study of 9 relapsed/refractory PCNSL patients, tumor specific ctDNA was found in the CSF in all patients [78]. Of note, in 3 patients, no CSF involvement could be detected using conventional MRI or CSF cytology and flow cytometry. At the time of tumor recurrence, between 11%-37% of single nucleotide variants found in the CSF were shared with the original tumor. The frequency of shared mutations (60%) was higher for mutations belonging to BCR pathway participants (e.g. *MYD88* L265P, *CD79B Y196*, CARD11). CSF was also collected through the course of treatment for ctDNA testing, and 7 out of 9 patients with repeated collections were observed to clear their CSF of ctDNA, which corresponded to response on brain imaging. One patient with early disease progression after initial tumor response had persistence of ctDNA in the CSF suggesting that ctDNA in the CSF may be a potential marker for minimal residual disease [78]. 1/3 cases with CD79B

Prognostic Markers

Two prognostic scoring systems have been used: 1) Memorial Sloan Kettering Cancer Center (MSKCC) prognostic score and 2) International Extranodal Lymphoma Study Group (IELSG) score. The MSKCC score separates patients into 3 groups stratified by age and Karnofsky performance status (KPS). Age ≤50 years correlated with a median overall survival (OS) of 8.5 years, age >50 years plus KPS ≥70 correlated with OS of 3.2 years, and age >50 years plus KPS <70 correlated with OS 0.9 years [79]. The IELSG score, on the other hand, scores patients based on age, Eastern Cooperative Oncology Group (ECOG) performance score, lactase dehydrogenase level, CSF protein concentration, and deep brain involvement. A score of 0-1 corresponds to a 2-year survival rate of 80%. A score of 2-3 corresponds to a 2-year survival rate of 48%, and a score of 4-5 corresponds to a 2-year survival rate of 15% [80].

In systemic DLBCL, the ABC (or non-GC) subtype has been associated with poor clinical outcome. Multiple studies have suggested that classifying PCNSL into GCB or non-GC does not predict a survival benefit. Raoux et al determined no significant survival difference between GCB and non-GC patients in 39 cases where 13 tumors were classified as GCB and 26 patients as non-GCB [81]. Similarly, Liu et al analyzed 89 cases with 18 tumors

classified as GCB and 71 as non-GCB without a difference in overall survival or PFS between the two groups [82]. Table 2 delineates multiple other studies that have examined survival difference between GCB and non-GC groups in PCNSL, all of which did not report a significant difference in overall survival [24, 81-86]. Limitations of these studies include small sample sizes, retrospective nature, and incomplete treatment information that could impact overall survival.

There has also been conflicting evidence regarding the prognostic significance of BCL6 protein detection by immunohistochemistry. A recent metanalysis of 22 studies involving 3037 DLBCL patients suggested that *BCL6* rearrangement portends a worse overall survival but does not seem to affect progression free survival (PFS) [87]. For PCNSL, the CALGB 50202 trial suggested that high *BCL6* expression correlated with shorter PFS, which was supported by a post-hoc analysis of the G-PCNSL-SG1 prospective trial [15, 88]. Other smaller studies, however, have suggested that BCL6 expression is associated with a better survival [89, 90].

CXCL13 has also been implicated as a poor prognostic marker in PCNSL patients perhaps due to its function of mediating pro-survival signals through B cell activation [91]. Rubenstein et al noticed that newly diagnosed patients with low CXCL13 levels in the CSF at time of diagnosis had a longer progression free survival with standard treatment compared to patients with high levels of CXCL13 [50]. CXCL13 has often been studied with IL-10 where the combination of elevated CXCL13 levels and IL-10 levels in the CSF provided increased specificity but decreased sensitivity for PCNSL detection. Nguyen-Them et al showed that serial CSF IL-10 measurements during treatment correlated with the course of the disease where the patients with a detectable level of IL-10 at the end of treatment despite imaging response were more likely to relapse in the first year compared to patients who had undetectable levels [68].

The recent genomic sequencing data has also not significantly contributed to novel prognostic biomarkers. Frequently mutated genes, like MYD88 or CD79B do not have prognostic value yet in PCNSL in contrast to systemic DLBCL where response to ibrutinib, a selective Bruton tyrosine kinase inhibitor, depends on the mutational status of these two genes. In systemic DLBCL, an overall response rate to ibrutinib was seen in 37% of ABC DLBCL and only 5% of GCB DLBCL, but the response was particularly high in those ABC DLBCL with a mutation in the BCR signaling pathway (55%) and coexisting mutations in both MYD88 and CD79B (80%). Those with MYD88 mutations but wild-type CD79b were unresponsive to ibrutinib [33]. These results however were not observed in PCNSL patients. Responses to ibrutinib were seen in both GCB and ABC PCNSL and also in tumors without mutations in the BCR pathway. Incomplete tumor responses were associated with mutations in the B-cell antigen receptor-associated protein CD79B. Based on the current data, only PCNSL patients with CARD11 mutations, which confer upfront resistance to BTK inhibition, should not be treated with ibrutinib [26].

Conclusion

There has been significant progress made in understanding the molecular pathogenesis of PCNSL leading to the use of targeted agents such as ibrutinib, a BTK inhibitor, for the treatment of PCNSL as well as agents targeting the activation of NF-kB, e.g. immunomodulatory drugs such as lenalidomide or pomalidomide [92]. A stereotactic biopsy and pathologic review will represent the diagnostic gold standard in PCNSL. Different markers have been evaluated, particularly in the CSF but have not yet been evaluated rigorously to warrant routine clinical use. Next generation sequencing of ctDNA isolated from CSF samples might represent a promising diagnostic biomarker but need to be evaluated in a more stringent setting.

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Key Points

- Stereotactic biopsy and pathologic review remain the diagnostic gold standard in PCNSL
- Clinical parameters (mainly age and performance status) are still the most significant prognostic parameters in PCNSL
- Subgroups as defined by the Hans immunohistochemical staining algorithm no not have a different clinical outcome in PCNSL
- Different CSF markers have been evaluated but not yet applied to routine clinical use
- Next generation sequencing of ctDNA isolated from CSF samples might represent a promising diagnostic biomarker

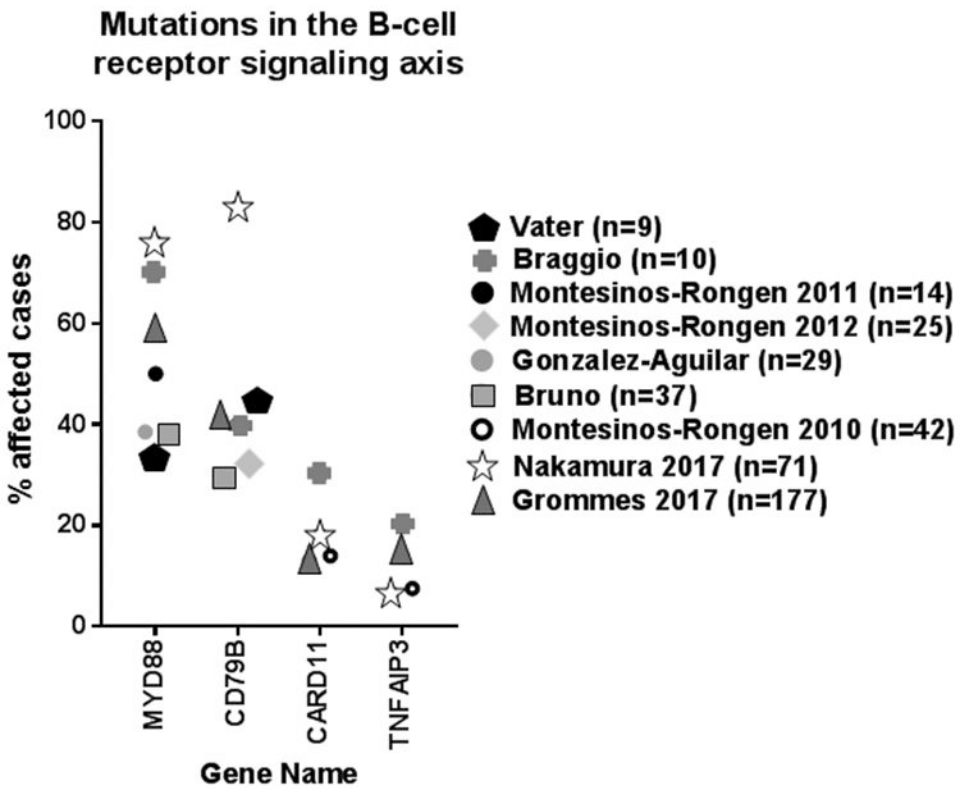

Figure 1. Frequent Mutations affecting the B-cell receptor signaling axis in PCNSL.

Mutations in *MYD88, CD79B, CARD11*, and *TNFAIP3* were found to be present in cases of PCNSL. The percentage of cases with mutations in these genes is delineated here with the majority of case studies reporting the presence of mutations within *MYD88*.

Table 1.

Potential diagnostic markers for primary central nervous system lymphoma (PCNSL).

AUC: area under the curve; CSF: cerebral spinal fluid; ddPCR: droplet digital PCR; ctDNA: circulating tumor DNA; NGS: next generation sequencing; PCR: polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; CBA: cytometric bead array; ECLIA: electrochemiluminescence immunoassay; CLEIA: chemiluminescent enzyme immune assay.

First Author (year)	No. Cases	Marker	*Serum or CSF*	Technology	AUC (95% CI)	Sensitivity	Specificity
DNA							
Hiemcke-Jiwa (2018)	32	*MYD88* p.(L265P)	CSF	ddPCR			
Hattori (2018)	14	*MYD88* p.(L265P)	CSF	ddPCR			
Fontanille (2017)	25	ctDNA	Serum	NGS			
		MYD88	Serum	NGS		24.0%	100.0%
RNA							
Baraniskin (2011)	23	*miR-21* + *miR-19b* + *miR-92a*	CSF	PCR		95.7%	96.7%
		miR-21	CSF	PCR		95.7%	83.3%
		miR-19b	CSF	PCR		95.7%	83.7%
		miR-92a	CSF	PCR		95.7%	80.0%
Mao (2014)	56	*miR-21*	serum	PCR	0.93 (0.88-0.98)		
Baraniskin (2016)	72	*RNU2-1f*	CSF	PCR	0.91	68.1%	91.4%
		RNU2-1f + *miR-21*	CSF	PCR	0.99	91.7%	95.7%
Chemokine							
Rubenstein (2013)	55	CXCL13	CSF	ELISA		71.0%	95.0%
		CXCL13 + IL-10	CSF	ELISA		50.0%	99.3%
Mabray (2016)	43	CXCL13	CSF	ELISA	0.83 (0.74-0.90)	76.7%	90.9%
Cytokine							
Rubenstein (2013)	55	IL-10	CSF	ELISA		64.0%	94.1%
Mabray	43	IL-10	CSF	ELISA	0.79 (0.69-0.87)	62.8%	95.5%
Nguyen-Them (2016)	112	IL-10	CSF	CBA	0.88	88.6%	88.9%
Sasagawa (2015)	19	IL-10	CSF	ELISA	0.97	94.7%	100.0%
Song (2016)	22	IL-10	CSF	ECLIA	0.96 (0.90-1.00)	95.5%	96.1%
		IL-6	CSF	ECLIA	0.61 (0.48-0.74)	54.6%	70.1%
		IL-8	CSF	ECLIA	0.56 (0.42-0.69)	31.8%	83.1%

First Author (year)	No. Cases	Marker	*Serum or CSF*	Technology	AUC (95% CI)	Sensitivity	Specificity
		TNFa	CSF	ECLIA	0.66 (0.41-0.68)	59.1%	57.1%
Sasayama (2012)	31	IL-10	CSF	ELISA	0.92 (0.84-0.99)	71.0%	100.0%
		IL-6	CSF	CLEIA	0.68 (0.57-0.79)	77.0%	63.0%
Protein Receptor							
Ikeguchi (2017)	12	sIL-2R	CSF	ELISA	0.87	83.3%	90.0%
Sasagawa (2015)	19	sIL-2R	CSF	ELISA	0.91	94.7%	84.6%
Sasayama (2012)	31	sIL-2R	CSF	ELISA	0.85 (0.75-0.96)	81.0%	56.7%
Thaler (2017)	33	sTACI	CSF	ELISA	0.94 (0.88-0.99)	87.9%	88.3%
		sBCMA	CSF	ELISA	0.76 (0.66-0.86)	72.7%	71.8%
Protein							
Strehlow (2016)	37	Osteopontin	CSF	ELISA	0.93 (0.87-1.00)	87.0%	86.0%
Sasagawa (2015)	19	B2M	CSF	Latex agglutination- turbidimetric immunoassay	0.81	89.4%	88.5%
Sasayama (2012)	31	B2M	CSF	Latex agglutination- turbidimetric immunoassay	0.93 (0.87-1.00)	88.0%	90.3%
Kuusisto (2015)	41	AT III	CSF	ELISA	0.79		
Roy (2008)	24	AT III	CSF	ELISA	0.91	75.0%	98.1%
Murase (1998)	12	sCD27	CSF	ELISA		100.0%	100.0%
Kersten (1996)	7	sCD27	CSF	ELISA		100.0%	98.0%
Viaccoz (2015)	28	Neopterin	CSF	Liquid chromatography with fluorimetric detection		96.0%	92.0%

Table 2.

Survival difference between diffuse large B-cell lymphoma subgroups

GCB: germinal center B-cell-like; non-GC: non-germinal center; NR: Not reported; HD-MTX: high-dose methotrexate (>3g/m^2); Ara-C: cytosine arabinoside; TMZ: temozolomide; RT: radiotherapy; CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone

First author (year)	No. cases	Median OS (months)	Median age (range)	Treatment
Liu (2017)	89	45.3	56 (11-85)	HD-MTX + Ara-C or HD-MTX + TMZ
GCB	18	*NR		
non-GC	71	*NR		
Aki (2013)	35	NR	52 (21-85)	Unspecified: some were treated with chemotherapy (NR) + RT or chemotherapy alone
GCB	6	19		
non-GC	29	17		
Hattab (2010)	31	NR	62 (13-81)	Most were treated with HD-MTX and steroids, but given the retrospective nature, some treatment regimens were unknown
GCB	5	2.5		
non-GC	26	7.4		
Raoux (2010)	39	5.3	67 (49-83)	19pts with chemotherapy and RT, 10pts with RT alone, 6pts with chemotherapy alone, 4pts untreated
GCB	13	*NR		
non-GC	26	*NR		
Momota (2010)	27	Not reached	63 (26-78)	All pts received HD-MTX; 25/27 pts received RT
GCB	1	N/A		
non-GC	22	N/A		
Lin (2006)	51	13.7	62 (16-82)	29 pts with HD-MTX, 1 pt with CHOP, 15 pts with RT, 6 pts with supportive care
GCB	7	34.5		
non-GC	22	11		
Camilleri-Broet (2006)	82	42	60 (23-80)	HD-MTX
GCB	3	*NR		
non-GC	79	*NR		