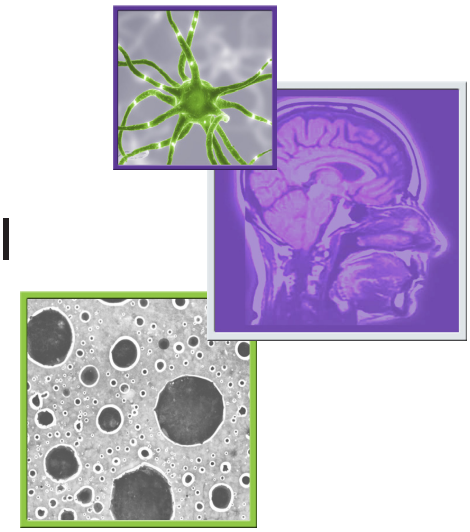
REVIEW

For reprint orders, please contact: reprints@futuremedicine.com

Modern cerebrospinal fluid analyses for the diagnosis of diffuse large B-cell lymphoma of the CNS



Alexander Baraniskin*[1] & Roland Schroers[1]

Practice Points

- Currently, the diagnosis of CNS lymphoma still represents a challenge, even with invasive diagnostic methods.
- Analysis of the cerebrospinal fluid (CSF) comprises an essential part of the diagnostic work-up for patients with suspected CNS lymphoma.
- Cytopathology is the basic examination of CSF with a low diagnostic accuracy.
- Flow cytometry is a reproducible method that allows the identification of clonal B-cell populations with high sensitivity.
- Diverse CSF proteins have been reported to be biomarkers for the diagnosis of CNS lymphomas, which need to be validated.
- PCR in association with direct sequence analysis of rearranged variable region genes is able to identify monoclonal B-cell populations.
- miRNAs are newly discovered small, noncoding, regulatory RNA molecules with outstanding stability and promising diagnostic accuracy, which needs to be validated.

SUMMARY CNS lymphomas represent rare and aggressive variants of extranodal non-Hodgkin's lymphomas, which may present with diverse neurological symptoms and are often diagnostically challenging. Primary CNS lymphomas develop within the CNS and characteristically involve the brain, leptomeninges, eyes and, in rare cases, spinal cord. Secondary CNS lymphomas are characterized by expansion of systemic lymphomas to the CNS. Multimodal investigation of cerebrospinal fluid (CSF) comprises an important component of the diagnostic work-up for patients with suspected CNS lymphomas. Cytopathological examination of the CSF is still regarded as the 'gold standard' for the diagnosis of leptomeningeal malignant disease. However, cytopathology has only a low sensitivity in detecting leptomeningeal lymphoma involvement. Modern technologies including proteochemical and immunophenotypic studies by flow cytometry, and molecular genetic analyses of CSF may increase sensitivity and specificity, therefore, facilitating the diagnosis of CNS lymphomas. This review gives an overview and discussion of the current aspects of CSF analyses in CNS lymphomas.

[1]Department of Medicine, Hematology & Oncology, Ruhr-University of Bochum, Germany
*Author for correspondence: Tel.: +49 234 299 0; Fax: +49 234 299 3459; alexander.baraniskin@rub.de

Future Medicine part of fsg

Classification & characteristics of CNS lymphoma

Primary lymphomas of the CNS (PCNSL) are aggressive tumors representing an uncommon subcategory of extranodal non-Hodgkin's lymphomas (NHLs). PCNSL involve the brain, leptomeninges, eyes and, infrequently, spinal cord. They affect both immunocompetent and immunocompromised patients. PCNSL occur without any systemic disease involvement at the time of diagnosis, and they have to be separated from secondary spread of systemic lymphomas to the CNS (secondary lymphomas of the CNS [SCNSL]). SCNSL are defined as NHL involvement both within and outside of the CNS at NHL diagnosis or at relapse of NHL.

PCNSL represent approxiamtely 3% of all brain tumors in immunocompetent patients [1]. The median age at diagnosis is between 60–65 years. Registry studies from different continents suggest that the incidence of PCNSL in immunocompetent patients is cumulatively rising [2]. The occurrence of secondary CNS involvement in aggressive lymphomas depends largely on the pathological subtype. CNS involvement is observed in 2–10% of immunocompetent patients with aggressive lymphomas histopathologically classified as diffuse large B-cell lymphomas during the course of disease [3]. In nearly all cases, the histologic subtype of PCNSL corresponds to the diffuse large B-cell lymphoma type; less than 4% of PCNSLs are of T-cell origin in western countries and of approximately 8% in Japan [4].

Early diagnosis is essential owing to the limited time frame to start potentially successful treatment of PCNSL and SCNSL. The clinical symptoms/signs of CNS lymphomas are not specific and do not allow separation of CNS lymphomas from important differential diagnoses, such as gliomas or inflammatory CNS diseases. The symptoms of CNS lymphoma include psychomotor slowing, cognitive dysfunction and personality changes. In addition, raised intracranial pressure and focal symptoms are also observed in a majority of PCNSL patients. However, headache, brainstem symptoms, cerebellar symptoms, cranial nerve dysfunction and seizures are only present in a minority [5]. In order to verify the suspected diagnosis, radiological and neurosurgical procedures are indispensable and represent the current standard of diagnostics. Despite recent advances in radiological techniques, the diagnosis of CNS lymphoma remains challenging. Although, stereotactic needle biopsies of CNS tumors are associated with low morbidity and mortality, this procedure is invasive, with diagnostic sensitivities ranging from 20 to 65% of biopsies in immunocompetent patients [6–8]. However, in our own experience, the rate of successful brain biopsies in CNS lymphomas is above 90% without noteworthy morbidity. An aggravating factor is that in certain clinical situations, depending on the location of the lesion, a stereotactic needle brain biopsy is not suitable. Furthermore, the frequently encountered corticosteroid application prior to the stereotactic biopsy significantly hampers a conclusive diagnosis. Hence, an important task of current research projects is to identify lymphoma markers in the CSF, which may facilitate the diagnosis of PCNSL and SCNSL.

Considering the improved prognosis of PCNSL in contrast to other primary malignant brain tumors as a result of novel and improved therapeutic strategies, PCNSL is of distinct interest in this context [9].

This review is focused on studies of CSF as a diagnostic tool for patients with suspected CNS lymphomas, predominantly PCNSL of diffuse large B-cell lymphoma type. An overview and discussion of current data on cytopathological, immunophenotypic and molecular genetic techniques in CSF analyses in CNS lymphoma are provided.

CSF analysis

The existence of CSF was first mentioned by Hippocrates of Cos (430 BC–370 BC) [10]. In 1890, more than 2000 years later, Heinrich Quincke, an internist from Kiel (Germany), performed the first percutaneous lumbar puncture [11–13]. In 1894, Freyhan was the first to report on cytological examination of CSF and its diagnostic value in suspected meningitis [14,15]. Oligoclonal immunoglobulin G autochthonously produced in CSF was described by Tourtelotte in 1970 [16] and free immunglobulin light chains in CSF were detected by Riberi in 1975 [17]. In 1990, successful PCR with cells obtained from CSF were reported [18]. The first flow cytometry analyses of cells in CSF were performed at the beginning of 1990s [19]. Finally, the first evidence for the presence of noncoding RNA molecules in CSF was provided in 2008 [20].

Today, multimodal investigation of the CSF comprises an important component of the diagnostic work-up for patients with suspected CNS

lymphoma. Although, there are no absolute contraindications to lumbar puncture, the indication should be carefully established owing to a risk of brain herniation, an uncommon but serious complication. Space occupying CNS lesions may result in increased intracranial pressure with descending transposition of the cerebrum and brainstem. Lumbar puncture that generally results in a minor, temporary dropping of lumbar CSF pressure may raise this pressure gradient and result in brain herniation [21]. Even though cytopathological examination of the CSF is still regarded as the 'gold standard' for the diagnosis of meningeal malignant disease [22], innovative technologies, including proteochemical, cellular immunophenotyping by flow cytometry and molecular genetic analyses of CSF, have successfully been advanced to improve the diagnosis of leptomeningeal affection in lymphoma and are becoming increasingly important.

Cytopathology

Leptomeningeal involvement is a common feature of PCNSL. Owing to distinct difficulties to prove leptomeningeal involvement noninvasively, its real prevalence is often underestimated. Onda *et al.* reported on the detection of leptomeningeal involvement in as many as 80% of patients in post-mortem histopathologies [23].

Unfortunately, the diagnostic accuracy and, particularly, the sensitivity of the cytopathology based exclusively on Pappenheim-stained cytospin specimens is limited [24]. Cytological characteristics of lymphoma cells are enlarged cell size, irregular shape and pointed margins of the cytoplasm, as well as deep notches in the nucleus [25]. In the majority of cases, additional immunocytochemistry is required to prove malignancy. In the four largest studies including more than 150 patients with PCNCL, leptomeningeal involvement was detected by cytomorphology in up to 16% of the patients [26–29]. The sensitivity of cytomorphological examination seems to be too low given the common periventricular location of CNS lymphomas and the high rate of leptomeningeal involvement in post-mortem histopathologies [22]. The most likely explanation for this apparent contradiction may be the low frequency and the fragility of the tumor cells in CSF in the changed conditions after lumbar puncture, and by prior use of corticosteroids [30]. The ratio of false-negative results may be reduced by increased volume of the CSF specimen to be analyzed (to 10.5 ml), by immediate processing of the sample and by repeating the lumbar puncture in cases of an initial negative cytological result [31].

False-positive results in cytomorphology probably arise by the morphological similarity of confounding reactive CSF lymphocytes, which are characteristically enlarged with blast-like nuclei as observed in lymphoma cells.

To conclude, cytopathology based on conventional staining methods only results in a low sensitivity in the detection of leptomeningeal lymphoma involvement. Therefore, additional immunocytochemistry methods are required.

Flow cytometry

Flow cytometry has significantly increased in importance for the diagnosis of many hematological malignancies in routine clinical practice. Flow cytometry is easily reproducible for the detection of malignant B lymphocytes, which can be separated from an abundance of reactive cells based on size, granularity and antigen expression profile [32,33]. French *et al.* reported an increase in the detection rate of 50% in the CSF by a combination of flow cytometry analysis and cytopathology, compared with cytopathology alone, in a retrospective study of 32 patients with lymphoproliferative disorders involving the CNS [34]. In another study published by Finn and colleagues, a similar increase of 43% in detection of malignant lymphoma in the CSF by multiparameter flow cytometry was reported. It is noteworthy, that flow cytometry allowed the identification of clonal B-cell populations comprising of less than 1% of total cells [32].

Regarding the detection of CSF involvement in PCNSL, application of flow cytometry also provides a superior diagnostic sensitivity compared with cytopathology alone. In our study, we compared cytopathology of conventionally stained slides with multiparameter flow cytometry in CSF specimens collected from 30 PCNSL patients. Here, we demonstrated a higher sensitivity of 23.3% for flow cytometry compared with a sensitivity of 13.3% for cytopathology [33]. Contradictory data were reported by Kiewe *et al.*, here a comparison of cytopathology with flow cytometry indicated a significantly higher sensitivity of cytopathology (11%) as compared with the sensitivity of flow cytometric immunophenotyping (3%) [30]. The conflicting results of both studies are best explained by technical differences, such as different flow cytometry protocols. In our study, both immunoglobulin

light chain restriction and expression of the cell surface marker CD10 were considered a criterion for malignant B lymphocytes in the CSF [33]. To better define the diagnostic value of immunophenotyping by flow cytometry in PCNSL, larger prospective studies are required.

Data on application of flow cytometric immunophenotyping of CSF cells in patients with SCNSL seem to be more valid compared with PCNSL due to the markedly larger patients numbers analyzed in published studies. In two of the largest studies, considerably augmented sensitivities of flow cytometry compared with cytopathology were demonstrated [35,36]. Hedge *et al.* reported a direct comparison of cytopathology and multicolour flow cytometry. CSF samples of 51 patients with newly diagnosed aggressive B-cell lymphoma at risk for CNS disease were analyzed with both methods. Occult CSF involvement was discovered in 11 (22%) patients by flow cytometry. However, cytopathology alone detected CSF involvement in only one of these patients. Quantitatively, flow cytometry enabled identification of B-cell clones that comprised as little as 0.2% of the total cellular compartiment. By comparison, the neoplastic cells had to constitute at least 5% of nucleated cells to be detectable by cytopathology [35]. In another study, Quijano *et al.* reported on a series of 123 patients with newly diagnosed aggressive B-cell lymphoma at risk of CNS involvement [36]. Corresponding to the results by Hegde *et al.*, a rise in sensitivity from 6 to 22% as determined by cytopathology was observed [36]. In two recently published studies, Stacchini *et al.* reported a CNS involvement in 24% of 62 samples analyzed with flow cytometry, whereas cytopathology found positive results in only 16% [37]. Benevolo *et al.* detected neoplastic population of B-NHL cells in CSF of 18 out of 174 (10%) patients, whereas cytopathology identified abnormal cells in only seven (4%) cases. The lower rate of positive cases observed in this study (10%) by flow cytometry was presumably due to the exclusion of patients with signs of neurologic disease, which were included in other studies [38].

Data from previous studies indicated that CSF samples often contain low cell numbers making flow cytometry analysis difficult. One explanation for this is the fragility of viable cells and their consecutive fast decline in native CSF. Nowadays, it is possible to increase the stability of cells in CSF for up to 10 days by applying cellular fixation using chemical stabilization methods [36,39]. Thus, logistical problems can be solved and the performance of large multicenter studies with uniform PCNSL patient CSF analyses in a central reference laboratory will be facilitated.

Protein markers

Different CSF proteins, including antithrombin III, soluble CD27, free immunoglobulin light chains, IL-10 and CXCL13 have been reported to be potentially useful biomarkers for the diagnosis of CNS lymphomas. However, their routine use in clinical practice of lymphoma diagnosis still remains to be established.

Using proteomic techniques Roy *et al.* identified antithrombin III, an acute phase response protein, as a novel CSF biomarker for CNS lymphoma. Measurements of antithrombin III concentration in CSF by ELISA allowed the separation of PCNSL from non-neoplastic controls with high accuracy (75% sensitivity and 98% specificity) [40]. Although the selective expression of antithrombin III in PCNSL with localization of the protein in tumor cells was demonstrated, it still remains an uncertainty whether the high CSF concentration of antithrombin III actually is derived from the lymphoma cells or results from protein leakage across the blood–brain barrier. Thus, antithrombin III would not be considered a specific lymphoma biomarker if its abundance in the CSF is caused by general leakage across the blood–brain barrier.

Kersten *et al.* reported that soluble CD27 (sCD27) levels in the CSF are raised in patients with meningeal spreading of lymphoid malignancies involving PCNSL [41,42]. CD27, a lymphocyte-specific TNF receptor superfamily member, is a transmembrane glycoprotein expressed on cell surface of T lymphocytes, natural killer cells, and normal and malignant B lymphocytes. CD27 expression is present in most B-cell malignancies at varying levels, and also in adult T-cell leukemias and lymphomas [43]. Kersten *et al.* revealed a remarkable sensitivity of 100% and specificity of 82% for sCD27 in recognition of leptomeningeal disease in acute lymphoblastic leukaemia and NHLs [41]. Although, there is some concern regarding the specificity of sCD27 measurements in CNS lymphomas owing to its increased expression both on activated T- and B-cells during infectious and inflammatory CNS diseases [44,45]. van den Bent *et al.* investigated CSF samples of patients and demonstrated a positive predictive

value of sCD27 determination for leptomeningeal involvement in B-cell malignancies of only 54% [46].

Another protein-based marker in CSF is IL-10. IL-10 is a pleiotropic cytokine excreted by normal and neoplastic B-lymphocytes [47]. Additionally, IL-10 has been described to be elevated in the serum of patients with aggressive, systemic NHL [48]. Sasayama *et al.* measured the IL-10 concentration in CSF of 26 patients with PCNSL and 40 patients with other brain tumors. A sensitivity of 71% and specificity of 100% were demonstrated. Furthermore, data of this study indicates an association between the elevated CSF level of IL-10 and a negative prognosis, and suggest IL-10 as a biomarker for the course of disease [49]. Two additional studies dealing with IL-10 as a marker in CSF with a lower sample size reported similar results [50,51].

Fischer *et al.* reported on CXCL13 in CSF as a biomarker for CNS lymphoma [52]. CXCL13 is a small cytokine that is selectively chemotactic for B lymphocytes. CXCL13 regulates the organization of B lymphocytes within the follicles of lymphoid tissues [53]. The authors evaluated concentrations of CXCL13 in CSF of 30 patients with CNS lymphoma and 40 controls including ten patients with brain tumors and 30 patients without malignancy and without an inflammatory disease. CXCL13 levels were significantly higher in patients with CNS lymphoma and correlated with a response to chemotherapy [52].

Rubenstein *et al.* recently published results of a large multicenter study containing 220 patients dealing with the abovementioned biomarkers, IL-10 and CXCL13. Here, the concentrations of CXCL13 and IL-10 in the CSF of CNS lymphoma patients (n = 83) and controls, including inflammatory disease (n = 71), brain tumors (n = 20) and patients without a CNS disease, were measured. Considering single markers a sensitivity of 69.9% and specificity of 92.7% for CXCL13 and a sensitivity of 65.4% and specificity of 92.6% for IL-10 were found. A combined bivariate method including the detection of increased levels of either CXCL13 or IL-10 allowed differentiation of patients with CNS lymphomas with a remarkable sensitivity of 84.2% and specificity of 90.5% [54].

The potential diagnostic value of κ- and λ-free immunoglobulin light chains (FLC) concentration/ratio in the CSF has currently been evaluated in two studies s [24,55]. Hildebrandt *et al.* demonstrated that FLC concentrations/ratios in the CSF are promising parameters for diagnostic usage in leptomeningeal lymphoma [55]. In our own study, we evaluated the role of FLC concentration and ratio in patients with CNS lymphoma. Irrespective of leptomeningeal lymphoma proven by cytopathology, we detected markedly aberrant FLC ratios in 52% (11 out of 21) of patients with CNS lymphomas compared with controls. Interestingly, an increased FLC ratio in CSF was preferentially detected in those patients with subependymal lymphoma growth as detected by MRI [24].

Quantification of conventional protein-based indices in CSF, such as total protein and β2-microglobulin, are considered sensitive and specific diagnostic methods for CNS lymphomas [36,56].

Molecular genetic markers

■ PCR of rearranged immunoglobulin genes

An exceptional feature of B cells is their ability to generate functional antigen receptors by somatic recombination of several gene segments. Within a normal immune response, B lymphocytes stimulated by antigens usually undergo somatic hypermutation in their immunoglobulin variable region genes creating a broad series of antibodies. B lymphocytes exhibiting a high affinity for a specific antigen are selectively rescued from apoptosis to differentiate into either antibody-producing plasma cells or circulating memory cells. Therefore, B-cell malignancies can be considered as B cells detained at a certain differentiation stage at the time that neoplastic transformation occured [57]. PCNSL display rearranged and somatically mutated immunoglobulin genes with distinct levels of intraclonal heterogeneity indicating ongoing mutation [58,59]. Therefore, PCR in association with direct sequence analysis of rearranged variable region genes seems to be a sensitive tool in identifying monoclonal B-cell populations. In cases in which cytomorphology fails to find a conclusive diagnosis, PCR analysis of immunoglobulin heavy and light chain genes may be advantageous. PCR may find a monoclonal B-cell population with somatically mutated immunoglobulin genes even in patients with corticoid-pretreated lymphoma.

PCR analysis of immunoglobulin heavy and light chain genes can also be applied to analyze CSF. In three studies, CSF samples of patients with PCNSL have been analyzed by means of PCR, and PCR results were compared with the

results of the cytomorphological examinations. Overall, the studies demonstrated contradictory results. Gleissner *et al.* [60] and Kiewe *et al.* [30] showed a distinct superiority of PCR with 8 and 11% of leptomeningeal involvement detected by cytomorphological examination and 11 and 16% by PCR, respectively. However, Fischer *et al.* described a higher sensitivity of cytomorphological examination (16%) compared with PCR (13%) [61]. Evaluating the results of the three studies the high rates of conflicting cytomorphological and PCR results need to be taken into account. One of the possible reasons for the high ratio of PCR false-negatives might be the corticosteroid pretreatment due to PCR failure happening more frequently in patients with corticosteroid therapy. This phenomenon may be caused by corticoid-induced loss of tumor cells due to apoptosis, which, resulted in a reduction of lymphoma cells even below the detection level of PCR [61,62].

■ miRNA

miRNAs are small, noncoding, regulatory RNA molecules that regulate the expression of their target mRNA predominantly by binding the 3′-untranslated regions of mRNA transcripts. miRNAs restrain gene expression at a post-transcriptional level by interfering with translation repression at the initiation or elongation stages, or by deadenylation resulting in degradation of mRNA [63,64].

Over the past several years, miRNAs have been shown to play important regulatory roles in a wide range of genetic pathways. Furthermore, it has become clear that alterations in the expression of miRNA genes contribute to the pathogenesis of many human malignancies, including leukemia and lymphoma [63].

Deregulated miRNA expression profiles were identified in many human cancers using miRNA profiling techniques on primary cancer tissue or on peripheral circulation, such as peripheral blood, and, interestingly, CSF. Cogswell *et al.* were the first to describe miRNA in CSF [20]. Regarding the role of miRNA in CSF as diagnostic biomarker for PCNSL, we recently demonstrated *miR-19b*, *miR-21* and *miR-92a* CSF levels, as determined by real-time PCR, to be significantly increased in PCNSL patients as compared to controls with inflammatory CNS disease or other neurologic disorders. *miR-21*, *miR-19b* and *miR-92a* had a significant diagnostic value for PCNSL, and, evaluation of these three miRNAs in combined expression analyses additionally improved their discriminatory diagnostic value. The combined *miR-21*, *miR-19b* and *miR-92a* analyses in PCNSL patients revealed that CSF levels of miRNA could distinguish, with a sensitivity of 95.7% and a specificity of 96.7%, patients with PCNSL from other neurologic disorders, most importantly from inflammatory CNS disease patients [65]. miRNAs are measurable in serum and are resistant to RNase digestion by ribonucleases in the serum. This stability of extracellular miRNAs circulating in the peripheral blood can be explained by embedding in protective cell membrane-derived particles, such as apoptotic bodies, microvesicles and exosomes. In agreement with these findings, miRNAs in the CSF demonstrated a remarkable stability as proved in exposure to exogenous RNase, repeated freeze–thaw cycles and long-term storage of CSF specimens [65]. Remarkably, the striking stability of CSF miRNAs as compared with the fragility of cells within the CSF characterizes an essential diagnostic advantage.

In a subsequent study, the role of the three previously mentioned miRNAs: *miR-21, miR-19b* and *miR-92* were validated in an enlarged PCNSL cohort (n = 39). Additionally, the role of CSF miRNAs as biomarkers for disease course monitoring was assessed. In sequential miRNA measurements in CSF originating from patients with different disease courses, a remarkable correlation of miRNA levels and PCNSL status during treatment and/or disease follow-up was demonstrated, that the levels of biomarker miRNAs, *miR-21, miR-19b* and *miR-92* decreased in patients with complete remission and increased in patients with refractory disease or who had relapsed [66].

Conclusion

Currently, the analysis of CSF is an established part of diagnosing PCNSL. This review focuses on the measurable alterations in CSF caused by PCNSL and highlights different methods to analyze CSF for the diagnosis of PCNSL and also SCNSL. Owing to an enormous amount of new knowledge and clinical studies, it is to be expected that the value of CSF diagnostics will increase with the advent of novel biomarkers, such as miRNAs and protein-based markers.

Future perspective

Today, cellular analyses of the CSF such as cytopathology and immunophenotyping by flow cytometry are the most important laboratory examinations when considering primary and secondary CNS lymphomas. Since sensitivities and specificities of these methods have been reported to be inappropriately low, novel techniques of CSF analyses are indispensable for noninvasive diagnosis of these diseases. We anticipate proteochemical and molecular genetic analyses, such as miRNA quantification of CSF, as promising diagnostic tools to facilitate the diagnosis and follow-up of CNS lymphomas in the near future.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:

- ■■ of considerable interest

1 Ney DE, Deangelis LM. Management of central nervous system lymphoma. In: *Non-Hodgkin Lymphomas.* Armitage JO, Mauch PM, Harris NL, Coiffier B, Dalla Favera R (Eds). Lippincott Willilams & Wilkins, PA, USA, 527–539 (2010).

2 Kluin PM, Deckert M, Ferry JA. Primary diffuse large B-cell lymphoma of the CNS. In: *WHO Classification of Tumours of Haemato-poietic and Lymphoid Tissues.* Swerdlow SH, Campo E, Harris NL *et al.* (Eds) WHO, Lyon, France, 240–241 (2008).

3 Ferreri AJ, Assanelli A, Crocchiolo R, Ciceri F. Central nervous system dissemination in immunocompetent patients with aggressive lymphomas: incidence, risk factors and therapeutic options. *Hematol. Oncol.* 27, 61–70 (2009).

4 Shenkier TN, Blay, JY, O'Neill BP *et al.* Primary CNS lymphoma of T-cell origin: a descriptive analysis from the international primary CNS lymphoma collaborative group. *J. Clin. Oncol.* 23, 2233–2239 (2005).

5 Korfel A, Schlegel U. Diagnosis and treatment of primary CNS lymphoma. *Nat. Rev. Neurol.* 9(6), 317–327 (2013).

6 Javedan SP, Tamargo RJ. Diagnostic yield of brain biopsy in neurodegenerative disorders. *Neurosurgery* 41, 823–828 (1997).

7 Josephson SA, Papanastassiou AM, Berger MS *et al.* The diagnostic utility of brain biopsy procedures in patients with rapidly deteriorating neurological conditions or dementia. *J. Neurosurg.* 106, 72–75 (2007).

8 Hall WA. The safety and efficacy of stereotactic biopsy for intracranial lesions. *Cancer* 82(9), 1749–1755 (1998).

9 Roth P, Martus P, Kiewe P *et al.* Outcome of elderly patients with primary CNS lymphoma in the G-PCNSL-SG-1 trial. *Neurology* 79(9), 890–896 (2012).

10 Arendt K, Demaerschalk BM, Wingerchuk DM, Camann W. Atraumatic lumbar puncture needles: after all these years, are we still missing the point? *Neurologist* 15(1), 17–20 (2009).

11 Pearce JM. Walter Essex Wynter, Quincke, and lumbar puncture. *J. Neurol. Neurosurg. Psychiatry* 57(2), 179 (1994).

12 Quincke HI. [About hydrocephalus]. *Verhandlung des Congress Innere Medizin* 10, 321–339 (1891).

13 Quincke HI. [The lumbar puncture]. *Berl. Klin. Wochenschr.* 28, 929–933 (1891).

14 Gray H. History of lumbar puncture. *Arch. Neurol. Psychiatry* 6(1), 61–70 (1921).

15 Zettl UK, Lehmitz R, Mix E. *[Clinical Diagnostics of Cerebrospinal Fluid]. (2nd Edition).* De Gruyter, Berlin, Germany (2005).

16 Tourtelotte WW. On cerebrospinal fluid immunoglobulin-G (IgG) quotients in multiple sclerosis and other diseases. A review and a new formula to estimate the amount of IgG synthesized per day by the central nervous system. *J. Neurol. Sci.* 10, 279–304 (1970).

17 Riberi M, Bernard D, Depieds R. Evidence for the presence of lambda chain dimers in cerebrospinal fluid of patients suffering from subacute sclerosing panencephalitis. *Clin. Exp. Immunol.* 19(1), 45–53 (1975).

18 Love S, Nicoll JA. The polymerase chain reaction and its applications in neuropathology. *Neuropathol. Appl. Neurobiol.* 18(2), 95–111 (1992).

19 Correale J, Mix E, Olsson T *et al.* $CD5^+$ B cells and CD4–8-T cells in neuroimmunological diseases. *J. Neuroimmunol.* 32(2), 123–132 (1991).

20 Cogswell JP, Ward J, Taylor IA *et al.* Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. *J. Alzheimers Dis.* 14(1), 27–41 (2008).

21 Scheld WM, Whitley RJ, Marra CM. *Infections of the Central Nervous System* (3rd Edition) Lippincott Williams & Wilkins PA, USA (2004).

22 Abrey LE, Batchelor TT, Ferreri AJ *et al.* Report of an international workshop to standardize baseline evaluation and response criteria for primary CNS lymphoma. *J. Clin. Oncol.* 23, 5034–5043 (2005).

■■ **One of the few reports about the prevalence of leptomeningeal involvement in post-mortem histopathology of patients with CNS lymphoma.**

23 Onda K, Wakabayashi K, Tanaka R, Takahashi H. Intracranial malignant lymphomas: clinicopathological study of 26 autopsy cases. *Brain Tumor Pathol.* 16, 29–35 (1999).

24 Schroers R, Baraniskin A, Heute C *et al.* Diagnosis of leptomeningeal disease in diffuse large B-cell lymphomas of the central nervous system by flow cytometry and cytopathology. *Eur. J. Haematol.* 85, 520–528 (2010).

25 Perske C, Nagel I, Nagel H, Strik H. CSF cytology-the ongoing dilemma to distinguish neoplastic and inflammatory lymphocytes. *Diagn Cytopathol.* 39(8), 621–626 (2011).

26 Korfel A, Weller M, Martus P *et al.* Prognostic impact of meningeal dissemination in primary CNS lymphoma (PCNSL): experience from the G-PCNSL-SG1 trial. *Ann. Oncol.* 23(9), 2374–2380 (2012).

27 Ferreri AJ, Blay JY, Reni M *et al.* Prognostic scoring system for primary CNS lymphomas:

the International Extranodal Lymphoma Study Group experience. *J. Clin. Oncol.* 21, 266–272 (2003).

28 Blay JY, Conroy T, Chevreau C *et al.* High-dose methotrexate for the treatment of primary cerebral lymphomas: analysis of survival and late neurologic toxicity in a retrospective series. *J. Clin. Oncol.* 16, 864–871 (1998).

29 Fischer L, Martus P, Weller M *et al.* Meningeal dissemination in primary CNS lymphoma: prospective evaluation of 282 patients. *Neurology* 71, 1102–1108 (2008).

30 Kiewe P, Fischer L, Martus P, Thiel E, Korfel A. Meningeal dissemination in primary CNS lymphoma: diagnosis, treatment, and survival in a large monocenter cohort. *Neuro-Oncology* 12, 409–417 (2010).

31 Glantz MJ, Cole BF, Glantz LK *et al.* Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results. *Cancer* 82(4), 733–739 (1998).

32 Finn WG, Peterson LC, James C, Goolsby CL. Enhanced detection of malignant lymphoma in cerebrospinal fluid by multiparameter flow cytometry. *Am. J. Clin. Pathol.* 110, 341–346 (1998).

33 Schroers R, Baraniskin A., Heute C *et al.* Detection of free immunoglobulin light chains in cerebrospinal fluids of patients with central nervous system lymphomas. *Eur. J. Haematol.* 85, 236–242 (2010).

34 French CA, Dorfman DM, Shaheen G, Cibas ES. Diagnosing lymphoproliferative disorders involving the cerebrospinal fluid: in- creased sensitivity using flow cytometric analysis. *Diagn. Cytopathol.* 23, 369–374 (2000).

▪▪ **One of the largest studies comparing flow cytometry with cytopathology in cerebrospinal fluid (CSF)of patients with lymphoma.**

35 Hegde U, Filie A, Little RF *et al.* High incidence of occult leptomeningeal disease detected by flow cytometry in newly diagnosed aggressive B-cell lymphomas at risk for central nervous system involvement: the role of flow cytometry versus cytology. *Blood* 105, 496– 502 (2005).

▪▪ **One of the largest studies comparing flow cytometry with cytopathology in the CSF of patients with lymphoma.**

36 Quijano, S., Lopez, A., Manuel, S.J. *et al.* Identification of leptomeningeal disease in aggressive B-cell non-Hodgkin's lymphoma: improved sensitivity of flow cytometry. *J. Clin. Oncol.* 27, 1462–1469 (2009).

37 Stacchini A, Aliberti S, Demurtas A, Benevolo G, Godio L. Ten antibodies, six colors, twelve parameters: a multiparameter flow cytometric approach to evaluate leptomeningeal disease in B-cell non-Hodgkin's lymphomas. *Cytometry B Clin. Cytom.* 82(3), 139–144 (2012).

38 Benevolo G, Stacchini A, Spina M *et al.* Final results of a multicenter trial addressing role of CSF flow cytometric analysis in NHL patients at high risk for CNS dissemination. *Blood* 120(16), 3222–3228 (2012).

39 Canonico B, Zamai L, Burattini S, Barnett D, Papa S. Evaluation of leukocyte stabilisation in TransFix-treated blood samples by flow cytometry and transmission electron microscopy. *J. Immunological Methods* 295, 67–78 (2004).

40 Roy S, Josephson SA, Fridlyand J. *et al.* Protein biomarker identification in the CSF of patients with CNS lymphoma. *J. Clin. Oncol.* 26, 96–105 (2008).

41 Kersten MJ, Evers LM., Dellemijn PL *et al.* Elevation of cerebrospinal fluid soluble CD27 levels in patients with meningeal localization of lymphoid malignancies. *Blood* 87, 1985–1989 (1996).

42 Murase S, Saio M, Takenaka K *et al.* Increased levels of CSF soluble CD27 in patients with primary central nervous system lymphoma. *Cancer Lett.* 132, 181–186 (1998).

43 van Oers MH, Pals ST, Evers LM *et al.* Expression and release of CD27 in human B-cell malignancies. *Blood* 82, 3430–3436 (1993).

44 Hintzen RQ, van Lier RA, Kuijpers KC *et al.* Elevated levels of a soluble form of the T cell activation antigen CD27 in cerebrospinal fluid of multiple sclerosis patients. *J. Neuroimmunol.* 35, 211–217 (1991).

45 Murase S, Saio M, Andoh H *et al.* Diagnostic utility of CSF soluble CD27 for primary central nervous system lymphoma in immunocompetent patients. *Neurological Res.* 22, 434–442 (2000).

46 van den Bent MJ, Lamers CH, van 't Veer MB, Sillevis Smitt PA, Bolhuis RL, Gratama JW. Increased levels of soluble CD27 in the cerebrospinal fluid are not diagnostic for leptomeningeal involvement by lymphoid malignancies. *Ann. Hematol.* 81(4), 187–191 (2002).

47 Mocellin S, Marincola FM, Young HA. Interleukin-10 and the immune response against cancer: a counterpoint. *J. Leukoc. Biol.* 78, 1043–1051 (2005).

48 Nacinovic-Duletic A, Stifter S, Dvornik S. Correlation of serum IL-6, IL-8 and IL-10 levels with clinicopathological features and prognosis in patients with diffuse large B-cell lymphoma. *Int. J. Lab. Hematol.* 30, 230–239 (2008).

49 Sasayama T, Nakamizo S, Nishihara M *et al.* Cerebrospinal fluid interleukin-10 is a potentially useful biomarker in immunocompetent primary central nervous system lymphoma (PCNSL). *Neuro Oncol.* 14(3), 368–380 (2012).

50 Salmaggi A, Eoli M, Corsini E *et al.* Cerebrospinal fluid interleukin-10 levels in primary central nervous system lymphoma: a possible marker of response to treatment? *Ann. Neurol.* 47, 137–138 (2000).

51 Whitcup SM, Stark-Vancs V, Wittes RE *et al.* Association of interleukin 10 in the vitreous and cerebrospinal fluid and primary central nervous system lymphoma. *Arch. Ophthalmol.* 115, 1157–1160 (1997).

52 Fischer L, Korfel A, Pfeiffer S *et al.* CXCL13 and CXCL12 in central nervous system lymphoma patients. *Clin. Cancer Res.* 15(19), 5968–5973 (2009).

53 Ansel KM, Ngo VN, Hyman PL *et al.* A chemokine-driven positive feedback loop organizes lymphoid follicles. *Nature* 406 (6793), 309–314 (2000).

▪▪ **Large multicenter study that describes CXCL13 and IL-10 in CSF as markers for CNS lymphoma.**

54 Rubenstein JL, Wong VS, Kadoch C *et al.* CXCL13 plus interleukin 10 is highly specific for the diagnosis of CNS lymphoma. *Blood* 121(23), 4740–4748 (2013).

55 Hildebrandt B, Muller C, Pezzutto A., Daniel P. Dorken B, Scholz C. Assessment of free light chains in the cerebrospinal fluid of patients with lymphomatous meningitis – a pilot study. *BMC Cancer* 7, 185 (2007).

56 Ernerudh J, Olsson T, Berlin G, von Schenck H. Cerebrospinal fluid immunoglobulins and beta 2-microglobulin in lymphoproliferative and other neoplastic diseases of the central nervous system. *Arch. Neurol.* 44(9), 915–920 (1987).

57 Berek C. The development of B cells and the B-cell repertoire in the microenvironment of the germinal center. *Immunol. Rev.* 126, 5 (1992).

58 Montesinos-Rongen M, Kuppers R, Schluter D. *et al.* Primary central nervous system lymphomas are derived from germinal-center B cells and show a preferential usage of the V4–34 gene segment. *Am. J. Pathol.* 155, 2077–2086 (1999).

59 Thompsett AR, Ellison DW, Stevenson FK, Zhu D. V(H) gene sequences from primary central nervous system lymphomas indicate derivation from highly mutated germinal center B cells with ongoing mutational activity. *Blood* 94, 1738–1746 (1999).

60 Gleissner B, Siehl J, Korfel A, Reinhardt R, Thiel E. CSF evaluation in primary CNS lymphoma patients by PCR of the CDR III IgH genes. *Neurology* 58, 390–396 (2002).

61 Fischer L, Martus P, Weller M *et al.* Meningeal dissemination in primary CNS lymphoma: prospective evaluation of 282 patients. *Neurology* 71, 1102–1108 (2008).

62 Baraniskin A, Deckert M, Schulte-Altedorneburg G, Schlegel U, Schroers R. Current strategies in the diagnosis of diffuse large B-cell lymphoma of the central nervous system. *Br. J. Haematol.* 156, 421–432 (2012).

63 Lawrie, C.H. MicroRNAs and haematology: small molecules, big function. *Br. J. Haematol.* 137, 503–512 (2007).

64 Croce, C.M. Causes and consequences of microRNA dysregulation in cancer. *Nat. Rev. Genet.* 10, 704–714 (2009).

■■ **Report about miRNAs in CSF as biomarkers for primary lymphomas of the CNS.**

65 Baraniskin, A., Kuhnhenn, J., Schlegel, U. *et al.* Identification of microR- NAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. *Blood* 117, 3140–3146 (2011).

66 Baraniskin A, Kuhnhenn J, Schlegel U, Schmiegel W, Hahn S, Schroers R. MicroRNAs in cerebrospinal fluid as biomarker for disease course monitoring in primary central nervous system lymphoma. *J. Neurooncol.* 109(2), 239–244 (2012).