



# Diagnostic markers for CNS lymphoma in blood and cerebrospinal fluid: a systematic review

Anouk van Westrhenen,<sup>1,2</sup>   
 Lisanne C. A. Smidt,<sup>1</sup> Tatjana Seute,<sup>1,3</sup>  
 Stefan Nierkens,<sup>1,4</sup> Abraham C. J.  
 Stork,<sup>1,3,5</sup> Monique C. Minnema<sup>1,6</sup> and  
 Tom J. Snijders<sup>1,3</sup> 

<sup>1</sup>University Medical Center Utrecht, Utrecht, The Netherlands, <sup>2</sup>Stichting Epilepsie Instellingen Nederland (SEIN), Heemstede, The Netherlands,

<sup>3</sup>Department of Neurology & Neurosurgery, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands, <sup>4</sup>Laboratory of Translational Immunology, Department Laboratory and Pharmacy, University Medical Center Utrecht, Utrecht, The Netherlands, <sup>5</sup>Department of Neurology, General Hospital Hietzing with Neurological Center Rosenhügel, Vienna, Austria and <sup>6</sup>Department of Haematology, Cancer Center, University Medical Center Utrecht, Utrecht, The Netherlands

Received 20 January 2018; accepted for publication 28 March 2018

Correspondence: Dr Tom J. Snijders, University Medical Center Utrecht, Brain Center Rudolf Magnus, Department of Neurology & Neurosurgery, Internal address G03.232, PO Box 85500, 3508 GA Utrecht, The Netherlands.  
 E-mail: t.j.snijders@umcutrecht.nl

Diagnostic delay is still a major problem in central nervous system (CNS) lymphoma (Haldorsen *et al*, 2005). Although empirical evidence for the advantages of early diagnosis (for survival or other outcome measures) is lacking, the rapidly progressive nature of the disease and the – potentially irreversible – consequences of progressive CNS symptoms have led to the recommendation to obtain diagnosis as rapidly as possible (Nayak *et al*, 2015).

Primary CNS lymphomas (PCNSLs) account for 2.2% of primary CNS tumours (Dolecek *et al*, 2012). The majority (95%) of PCNSLs are histologically classified as a type of diffuse large B cell lymphoma (DLBCL) and the remaining are diverse such as Burkitt, lymphoblastic, marginal zone or T cell lymphomas (Ferrerri & Marturano, 2012). The highest

## Summary

Diagnosing central nervous system (CNS) lymphoma remains a challenge. Most patients have to undergo brain biopsy to obtain tissue for diagnosis, with associated risks of serious complications. Diagnostic markers in blood or cerebrospinal fluid (CSF) could facilitate early diagnosis with low complication rates. We performed a systematic literature search for studies on markers in blood or cerebrospinal fluid for the diagnosis CNS lymphoma and assessed the methodological quality of studies with the Quality Assessment of Diagnostic Accuracy Studies tool (QUADAS-2). We evaluated diagnostic value of the markers at a given threshold, as well as differences between mean or median levels in patients *versus* control groups. Twenty-five studies were included, reporting diagnostic value for 18 markers in CSF (microRNAs -21, -19b, and -92a, RNU2-1f, CXCL13, interleukins -6, -8, and -10, soluble interleukin-2-receptor, soluble CD19, soluble CD27, tumour necrosis factor- $\alpha$ , beta-2-microglobulin, antithrombin III, soluble transmembrane activator and calcium modulator and cyclophilin ligand interactor, soluble B cell maturation antigen, neopterin and osteopontin) and three markers in blood (microRNA-21 soluble CD27, and beta-2-microglobulin). All studies were at considerable risk of bias and there were concerns regarding the applicability of 15 studies. CXCL-13, beta-2-microglobulin and neopterin have the highest potential in diagnosing CNS lymphoma, but further study is still needed before they can be used in clinical practice.

**Keywords:** central nervous system (CNS) lymphoma, diagnostic marker(s), blood, cerebrospinal fluid (CSF), liquid biopsy.

rate of secondary CNS lymphoma (SCNSL) involvement (27%) is found in lymphoblastic lymphomas and Burkitt lymphomas (van de Schans *et al*, 2012). Intensive treatment with corticosteroids, chemotherapy and radiotherapy may be curative or offer effective (and sometimes long-lasting) palliation in patients with PCNSL who are in fair to good clinical condition (Morris & Abrey, 2009). Tumour resection through neurosurgery does not result in therapeutic benefit; hence, only biopsy is indicated to obtain a tissue diagnosis (Bellinzona *et al*, 2005). Clinical and radiological features may suggest the diagnosis of PCNSL, but are not diagnostic; in a typical clinical situation, a wide differential diagnosis will be considered, including other malignancies, demyelinating disease (multiple sclerosis) and neurosarcoidosis (Küker

*et al*, 2005; Zacharia *et al*, 2008; Ricard *et al*, 2012). Secondary CNS lymphoma typically presents with leptomeningeal, subependymal or cranial nerve contrast enhancement, which can also be seen in infections or inflammatory disease (Halvorsen *et al*, 2011).

Brain biopsy is the gold standard for the diagnosis of CNS lymphoma. This invasive procedure has a complication rate of 8.5%, consisting of haematomas, seizures or brain oedema, and biopsy-related mortality occurs in 1% (Khatab *et al*, 2014). Less invasive diagnostic options are cytology or flow cytometry of cerebrospinal fluid (CSF) or vitreous fluid (Scott *et al*, 2013). However, these are only positive in case of leptomeningeal or ocular involvement respectively. Also, the finding of a monoclonal population of lymphocytes may rarely be a false-positive result, because other diseases may also cause such a response (Vafaii & DiGiuseppe, 2014). Often steroid therapy is initiated to reduce symptoms before pathological diagnosis is obtained. The effects of corticosteroids, however, also make it more difficult to obtain a definite (histopathological) diagnosis, with an increased chance of false-negative findings (Roth *et al*, 2010).

Therefore, there is need for a diagnostic test with a high diagnostic yield and limited risks, leading to shorter time to diagnosis. A diagnostic marker obtainable from blood or CSF would fulfil this need, serving as a type of 'liquid biopsy'. For that reason, we performed a systematic and critical review of the literature on possible diagnostic markers for the diagnosis of central nervous system lymphoma.

## Methods

We performed a systematic literature search in PubMed using synonyms for (i) CNS lymphoma, (ii) blood or CSF, and (iii) markers. Articles were selected for title, abstract and full text, based on the inclusion and exclusion criteria presented in Fig 1. There were insufficient data for a quantitative meta-analysis of any given diagnostic marker and therefore a systematic descriptive analysis of the available data was performed. This review was written in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher *et al*, 2009).

We assessed the quality of all included studies using the 2011 revised version of 'Quality Assessment of Diagnostic Accuracy Studies' (QUADAS-2) (Whiting *et al*, 2011). The QUADAS-2 is designed for grading individual diagnostic studies on their risk of bias and applicability. As recommended in QUADAS-2, we adapted signalling questions to our review question. We omitted the signalling questions about blinding of the reference and index test, because the results of these tests are not at great risk for bias; the results consist of quantitative or dichotomous outcomes with little room for interpretation. Concerns regarding applicability were scored 'high' when the control group did not consist of patients with a clinical picture suggestive of CNS lymphoma, as this does not correspond to our review question. On the

basis of the quality assessment of the included studies, we determined the risk of bias and applicability of each individual biomarker. When classifications of risk of bias or applicability differed among different studies, we used the risk assessment of the most important study on this biomarker, based on sample size and quality of the study.

## Results

The search query in PubMed yielded 918 results, of which 25 studies were included (Fig 1). These studies showed a diagnostic value for levels of certain markers in blood or CSF in the diagnosis of primary or secondary CNS lymphoma. Studies that did not investigate diagnostic value but did find significant differences in levels of a marker between patient (CNS lymphoma) and control groups, were also included. The included studies concern the following markers: micro RNA (miR)-21, miR-19b, miR92a, U2 small nuclear RNA fragments (RNU2-1f), C-X-C motif chemokine Ligand 13 (CXCL13), interleukin (IL)-6, IL-8, IL-10, soluble IL-2 receptor (sIL-2R), soluble CD19 (sCD19), soluble CD27 (sCD27), tumour necrosis factor alpha (TNF- $\alpha$ ), beta-2-microglobulin (B2M), antithrombin III (ATIII), soluble transmembrane activator and calcium modulator and cyclophilin ligand interactor (sTACI), soluble B cell maturation antigen (sBCMA), neopterin and osteopontin.

### Markers

The diagnostic values of markers, as derived from the included studies, are listed for PCNSL (Table I) (Kersten *et al*, 1996; Murase *et al*, 2000; Roy *et al*, 2008; Baraniskin *et al*, 2011, 2012a, 2016; Sasayama *et al*, 2012; Rubenstein *et al*, 2013; Mao *et al*, 2014; Kuusisto *et al*, 2015; Sasagawa *et al*, 2015; Viacoz *et al*, 2015; Mabray *et al*, 2016; Nguyen-Them *et al*, 2016; Song *et al*, 2016; Strehlow *et al*, 2016; Ikeguchi *et al*, 2017; Thaler *et al*, 2017) and for SCNSL (Table II) (Ernerudh *et al*, 1987; Kersten *et al*, 1996; Kara *et al*, 2007; Muñiz *et al*, 2014). Some of the included articles reported the diagnostic values for PCNSL and SCNSL as one patient group (Roy *et al*, 2008), these results are also included in Table I. Mean or median levels of markers in CNS lymphoma versus control patients are presented in Table III. Details of patient and control group of each study can be found in supplementary Table I. The following paragraphs summarise the results for each marker.

### microRNAs and small nuclear RNAs

Baraniskin *et al* (2011) were the first to study the diagnostic value of microRNA levels in CSF of PCNSL patients. They found miR-21, miR-19b and miR-92a to be diagnostic markers for CNS lymphoma and suggested that a diagnostic tree should be implemented, starting with testing for miR-21 elevation, followed by testing for miR-19 or miR-92a elevation.

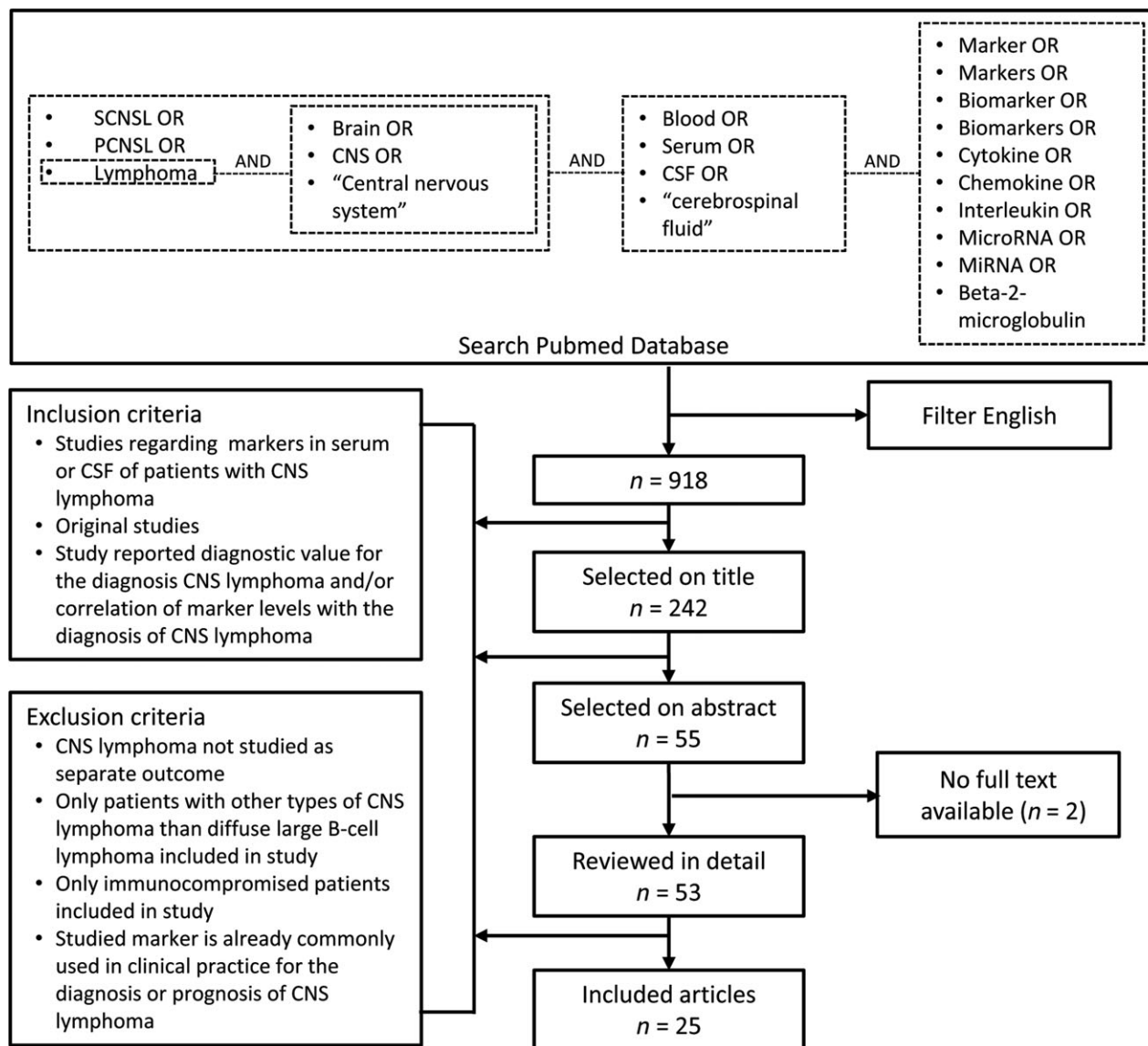


Fig 1. Flowchart of search in PubMed database. SCNSL, secondary central nervous system lymphoma; PCNSL, primary central nervous system lymphoma; CNS, central nervous system; CSF, cerebrospinal fluid.

This diagnostic tree had a sensitivity of 95.7% and a specificity of 96.7%. An expanded study group even yielded a slightly better sensitivity, of 97.4% (Baraniskin *et al*, 2012a). In this second study, the value of microRNAs in serum was also investigated in a small patient group, but no significant difference in serum microRNAs between PCNSL patients and control patients was found. Another study however did show a high diagnostic value of serum miR-21 for PCNSL (area under curve [AUC] 0.930) and showed an effective distinction between PCNSL and glioblastomas (AUC 0.883) (Mao *et al*, 2014). A correlation between miR-21 in serum and CSF was found in this study (Pearson correlation:  $r^2 = -0.396$   $P = 0.001$ ). A more recent study combined CSF marker miR-21 with small nuclear RNA fragments of RNU2-

1f in CSF (Baraniskin *et al*, 2016). They found a specificity of 95.7% for discriminating PCNSL patients from controls, with a sensitivity of 91.7% and an AUC of 0.987.

### CXCL13

The chemokine CXCL13 has an important role in the homing of B cells (Ansel *et al*, 2002). Three studies analysed CXCL13 as a marker in CNS lymphoma. A relatively small study compared the value of CXCL13 in CSF of 29 patients with a combined group of PCNSL and SCNSL patients, and control groups with and without other CNS malignancies (Fischer *et al*, 2009). They reported no sensitivity or specificity but showed a significant difference in

Table 1. Diagnostic value of markers in PCNSL (combined with SCNSL).

Study	Retrospective or prospective	Patient group		Control group		Marker	Serum or CSF	Cut-off	AUC [95% CI]	Sens (%)	Spec (%)
		Diagnosis (n)	N	Diagnosis (n)	N						
Ikeguchi <i>et al</i> (2017)	Retrospective	PCNSL (8) and SCNSL (4)	12	Multiple Sclerosis (64), NMOSD (35), TDL (17)	116	sIL-2R	CSF		0.87	83.3	90.0
		PCNSL*	33	Primary brain tumour (20), secondary brain tumour (22), neuroinfectious disease (13), neuroinflammatory disease (30), other neurological diseases (52)	137	IL-10 sTACI sBCMA sTACI + sBCMA	CSF	>68.4 pg/ml >460 pg/ml >68.4 pg/ml; >460 pg/ml	0.94 [0.88-0.99] 0.76 [0.66-0.86]	87.9 72.7	88.3 71.8
Baraniskin <i>et al</i> (2016)	Retrospective	PCNSL	72	CNS inflammation (35), miscellaneous neurological disorders (12)	47	RNU2-1f miR-21	CSF		0.91 0.99	68.1 91.7	91.4 95.7
		PCNSL (38) and SCNSL (5)	43	Metastases (21), high-grade gliomas (14), tumefactive demyelinating lesions (9)	44	CXCL-13 IL-10	CSF	>103.0 pg/ml >21.77 pg/ml	0.83 [0.74-0.90] 0.79 [0.69-0.87]	76.7 62.8	90.9 95.5
Nguyen-Thiem <i>et al</i> (2016)	Prospective	PCNSL	112	Glioma (9), ependymoma (2), medulloblastoma (2), brain metastasis (3), neuroinflam-matory disease (18), neuroinfectious disease (3), neurodegenerative disease (3)	40	IL-10	CSF	>4 pg/ml	0.88	88.6	88.9
Song <i>et al</i> (2016)	Retrospective	PCNSL	22	Systemic NHL with high risk of CNS involvement (41), CNS inflammatory disease (14), CNS infection (13), CNS demyelinating disease (5), other brain tumours (7)	80	IL-10 IL-6 IL-8 TNF- $\alpha$ IL-10/IL-6 ratio	CSF	>8.2 pg/ml >5.1 pg/ml >117 pg/ml >5.3 pg/ml >0.72	0.96 [0.90-1.00] 0.61 [0.48-0.74] 0.56 [0.42-0.69] 0.66 [0.41-0.68] 0.98 [0.93-1.00]	95.5 54.6 31.8 59.1 95.6	96.1 70.1 83.1 57.1 100
		PCNSL (29) and SCNSL (8)	37	Inflammatory CNS disease (6), Multiple sclerosis (8), glioblastoma (9), healthy controls (13)	36	Osteopontin	CSF	>400 ng/ml	0.93 [0.87-1.00]	87	86
		PCNSL (5), SCNSL (7) and systemic lymphoma (29)	41	Alzheimer disease (19), Multiple Sclerosis (6), no neurological disease (17)	42	Antithrombin III Albumin AT III/albumin ratio	CSF		0.79 0.84 0.25		
		PCNSL (5), SCNSL (7) and systemic lymphoma (29)	41	Alzheimer disease (19), Multiple Sclerosis (6), no neurological disease (17)	42	Antithrombin III Albumin AT III/albumin ratio	CSF		0.79 0.84 0.25		

Table 1. (Continued)

Study	Retrospective or prospective	Patient group		Control group		Marker	Serum or CSF	Cut-off	AUC [95% CI]	Sens (%)	Spec (%)																					
		Diagnosis (n)	N	Diagnosis (n)	N																											
Sasagawa et al (2015)	Retrospective	PCNSL (15) and SCNSL (4)	19	Glioblastoma (5), anaplastic astrocytoma (2), glioma (1), ependymoma (1), metastasis (5), immature teratoma (1), atypical meningioma (1), undifferentiated sarcoma (1), Multiple Sclerosis (3), other (6)	26	IL-10 IL-10/IL-6 ratio sIL-2R B2M	CSF	>3 pg/ml >2.2 >60.4 u/ml >2.4 mg/l	0.97 0.95 0.91 0.81	94.7 68.4 94.7 89.4	100 96.1 84.6 88.5																					
												Viaccoz et al (2015)	Retrospective	PCNSL	28	Other space occupying brain lesions (glioma (36), metastasis (13), other type of brain tumour (5), pseudotumoural inflammatory brain lesion (13)), nontumefactive inflammatory CNS disorders (29)	96	Neopterin	CSF	>10 nmol/l	96	92										
																							Mao et al (2014)	Retrospective	PCNSL	56	Other CNS malignancy (55), CNS inflammation (20), healthy (47)	122	miR-21	Serum	0.93 [0.88-0.98]	
	PCNSL HIV negative	55	Neuro-inflammation (71), primary brain tumour (8), brain metastasis (12), neoplasm/infection outside CNS (46)	137	CXCL13 IL-10 CXCL13 or IL-10 CXCL13 and IL-10	CSF CSF CSF CSF	>116 pg/ml >23 pg/ml >116 pg/ml; >23 pg/ml >116 pg/ml; >23 pg/ml	0.84 [0.78-0.89] 0.83 [0.79-0.90] 0.87 [0.82-0.92] 0.75 [0.68-0.81]	71 64 84.2 50	95 94 90.5 99.3																						

Table 1. (Continued)

Study	Retrospective or prospective	Patient group		Control group		Marker	Serum or CSF	Cut-off	AUC [95% CI]	Sens (%)	Spec (%)
		Diagnosis (n)	N	Diagnosis (n)	N						
Baraniskin <i>et al</i> (2012a)	Retrospective	PCNSL	39	CNS inflammation, other	37	miR-21 and miR-19b and/or miR-92a	CSF	>8.0 REL; >1.4 REL; >2.5 REL;	0.92 [0.84-0.99]	71	100
Sasayama <i>et al</i> (2012)	Retrospective + prospective part	PCNSL	31	Other CNS malignancy (57), CNS inflammation (2)	59	IL-10 IL-6 B2M sIL-2R	CSF	9.5 pg/ml 4.0 pg/ml 2056 µg/l 77 u/ml	0.68 [0.57-0.79] 0.93 [0.87-1.00] 0.85 [0.75-0.96]	77 88	63 90.3
Baraniskin <i>et al</i> (2011)	Retrospective	PCNSL	23	CNS inflammation (20), other (10)	30	miR-21 miR-19b miR-92a miR-21 and miR-19b and/or miR-92a	CSF	>8.0 REL >1.4 REL >2.5 REL >8.0 REL; >1.4 REL; >2.5 REL	0.94 [0.87-1.00] 0.98 [0.91-1.00] 0.97 [0.93-1.00]	95.7 87.7 80	83.3 87.7 96.7
Roy <i>et al</i> (2008)	Retrospective	PCNSL and SCNSL†	24	Malignancy outside CNS (29), CNS inflammation (15), other (33)	77	ATIII	CSF	1.2 µg/l	0.91	75	98.7
Murase <i>et al</i> (1998)	Retrospective	PCNSL	12	Other CNS malignancy	30	sCD27	CSF	15 U/ml		100	100
Kersten <i>et al</i> (1996)	Retrospective	PCNSL‡	7	Malignancy outside CNS, other CNS malignancy, PCNSL in remission (1)	41	sCD27	CSF	>10 U/ml		100	98

PCNSL, primary central nervous system lymphoma; SCNSL, secondary central nervous system lymphoma; NMOSD, neuromyelitis optica spectrum disorder; TDL, tumefactive demyelinating lesions; NHL, non-Hodgkin lymphoma; AUC, area under the curve; CI, confidence interval; sens, sensitivity; spec, specificity; REL, expression of miRNA relative to miR-24. For the investigated markers, abbreviations are used; see main text for their full names.

\*Patient group included 6 SCNSL patients, however diagnostic value only examined for PCNSL patients.

†Numbers not specified.

‡These are 8 samples from 4 PCNSL patients (one sample after treatment, when PCNSL was in remission) and 40 samples from 32 patients with other CNS malignancies (13) or malignancy outside CNS (19).



Table II. Diagnostic value of markers in SCNSL.

Study	Retrospective or prospective	Patient group		Control group		Marker	Serum or CSF	Cut-off	AUC; P-value	Sens	Spec
		Diagnosis (n)	N	Diagnosis (n)	N						
Muñiz et al (2014)	Retrospective	SCNSL	(40)*	DLBCL and BL without CNS involvement (40)*	CD19	CSF	>1.18 ng/ml	0.88 P < 0.05	80%	95%	
Kara et al (2007)	Retrospective	CNS localization of ALL (3), NHL (1), AML (1)	5	ALL (15), NHL (6), AML (4), epidural haemorrhage (2), lumbar disc protrusion (3)	B2M sCD27	CSF CSF	>2.56 ng/ml >350 u/ml	0.78 P < 0.05	62%	90%	
Kersten et al (1996)	Retrospective	SCNSL/CNS localization of ALL	(104)†	NHL and ALL without CNS involvement (children)	sCD27	CSF	>10 u/ml	0.99	83%	86%	
		SCNSL/CNS localization of ALL	(70)‡	NHL and ALL without CNS involvement	sCD27	CSF	>10 u/ml;	0.95	100%	82%	
		SCNSL/CNS localization of ALL	(38)‡	NHL and ALL without CNS involvement	sCD27 CSF/serum ratio	CSF/serum	>0.06	0.91	83%	88%	
		SCNSL/CNS localization of ALL	(54)‡	NHL and ALL without CNS involvement	B2M	CSF	>1.6 mg/l	0.77	81%	68%	
		SCNSL/CNS localization of ALL	(36)‡	NHL and ALL without CNS involvement	B2M CSF/serum ratio	CSF/serum	>0.7	0.82	92%	63%	
Emerudh et al (1987)	Retrospective	SCNSL	14	NHL and HD without CNS involvement	B2M	CSF	>1.9 mg/l		71%	67%	

SCNSL, secondary central nervous system lymphoma; DLBCL, diffuse large B cell lymphoma; BL, Burkitt lymphoma; NHL, non-Hodgkin lymphoma; ALL, acute lymphatic leukemia; AUC, area under the curve; sens, sensitivity; spec, specificity.

\*Number of patients in patients group plus control group.

†Data are based on 104 samples of 70 children with ALL/NHL undergoing routine CNS staging.

‡Data are based on 70 samples of 45 patients with ALL/NHL suspected of meningeal localization, sCD27 CSF/serum ratio, B2M and B2M CSF/serum ratio were only measured in a part of these samples.

Table III. Mean or median values of markers.

Study	Retro- or prospective	Marker	Serum or CSF	Patient group			Control group			P-value
				Diagnosis	N	Mean ± SD or median (range)	Diagnosis (n)	N	Mean ± SD or median (range)	
Ikeguchi <i>et al</i> (2017)	Retrospective	sIL-2R IL-10	CSF CSF	PCNSL + SCNSL	12	120 U/ml (71–330 U/ml) 5.5 pg/ml (≤2.0–13.0 pg/ml)	Multiple Sclerosis (64), NMOSD (35), TDL (17), glioma (10)	126	<54.5 U/ml ≤2.0 pg/ml	P < 0.001 P < 0.01
Thaler <i>et al</i> (2017)	Prospective	sTACI sBCMA	CSF Serum CSF	PCNSL SCNSL PCNSL PCNSL PCNSL PCNSL	33 6 21 33 6 21	445 pg/ml (IQR 1883 pg/ml) 32 pg/ml (IQR 3583 pg/ml) 348 pg/ml (IQR 289 pg/ml) 760 pg/ml (IQR 1740 pg/ml) 430 pg/ml (IQR 3095 pg/ml) 15.4 ng/ml (IQR 11.2 ng/ml)	Primary brain tumour (20), secondary brain tumour (22), neuroinfectious disease (13), neuroinflammatory diseases (30), other neurological diseases (52)	137	0 pg/ml (IQR 7 pg/ml) 338 pg/ml (IQR 291 pg/ml) 290 pg/ml (IQR 320 pg/ml)	NS NS
Baraniskin <i>et al</i> (2016)	Retrospective	RNU2-1f	Serum CSF Serum	PCNSL PCNSL PCNSL	72	24.78 ± 0.32 Ct values 22.01 ± 0.43Ct values	CNS inflammation (35), miscellaneous neurological disorders (12)	47	15.5 ng/ml (IQR 6.4 ng/ml) 29.07 ± 0.33Ct values 21.34 ± 0.64 Ct values	NS P < 0.0001 NS
Mabray <i>et al</i> (2016)	Retrospective	CXCL-13 IL-10	CSF CSF	PCNSL + SCNSL	43	2960.49 pg/ml (95% CI 1124.96–4796.01 pg/ml) 557.48 pg/ml (95% CI 167.49–947.47 pg/ml)	Metastases (21), high-grade gliomas (14), tumefactive demyelinating lesions (9)	44	72.54 pg/ml (95% CI 9.02–136.05 pg/ml) 5.93 pg/ml (95% CI 3.42–8.43)	P < 0.001 P < 0.001
Nguyen-Them <i>et al</i> (2016)	Prospective	IL-10	CSF	PCNSL*	112					
Song <i>et al</i> (2016)	Retrospective	IL-10	CSF	PCNSL	22	74.7 pg/ml (<5.0–1000 pg/ml)	High-risk systemic NHL CNS inflammatory disease CNS demyelinating disease CNS infection	41	<5.0 pg/ml (<5.0–197.0 pg/ml) <5.0 pg/ml <5.0 pg/ml	P < 0.0001 PCNSL vs. all others
		IL-6	CSF	PCNSL	22	5.2 pg/ml (<2.0–109.0 pg/ml)	Other brain tumours High-risk systemic NHL CNS inflammatory disease CNS demyelinating disease CNS infection	7 41 14 5 13	<5.0 pg/ml (<5.0–231.0 pg/ml) <5.0 pg/ml (<5.0–7.9 pg/ml) 2.5 pg/ml (<2.0–8.2 pg/ml) 3.2 pg/ml (<2.0–17.8 pg/ml) 2.2 pg/ml (<2.0–3.9 pg/ml) 271.0 pg/ml (<2.0–1000 pg/ml)	P < 0.01 PCNSL vs. high-risk systemic NHL
		IL-8	CSF	PCNSL	22	89.5 pg/ml (25.0–208.0 pg/ml)	Other brain tumours High-risk systemic NHL CNS inflammatory disease CNS demyelinating disease CNS infection	7 41 14 5 13	7.3 pg/ml (6.4–70.8 pg/ml) 61.5 pg/ml (28.0–129 pg/ml) 78.0 pg/ml (43.0–116.0 pg/ml) 39.0 pg/ml (35.0–50.0 pg/ml) 232.0 pg/ml (22.0–7500 pg/ml)	
		TNF-α	CSF	PCNSL	22	5.7 pg/ml (4.5–10.3 pg/ml)	Other brain tumours High-risk systemic NHL CNS inflammatory disease CNS infection	7 41 14 5	96.0 pg/ml (17.0–312.0 pg/ml) 5.0 pg/ml (<4.0–7.1 pg/ml) 5.7 pg/ml (4.8–6.7 pg/ml) 4.1 pg/ml (<4.0–5.0 pg/ml)	
		IL-10/IL-6 ratio	CSF	PCNSL	22	12.6 (0.00–114.9)	Other brain tumours High-risk systemic NHL CNS inflammatory disease CNS demyelinating disease CNS infection	7 41 14 5 13	4.8 pg/ml (4.2–15.1 pg/ml) 0.0 (0.0–40.2) 0.0 (0.0–0.0) 0.0 (0.0–0.0) 0.0 (0.0–0.2)	P < 0.0001 PCNSL vs. all other diseases except other brain tumours



Table III. (Continued)

Study	Retro- or prospective	Marker	Serum or CSF	Patient group			Control group			P-value
				Diagnosis	N	Mean ± SD or median (range)	Diagnosis (n)	N	Mean ± SD or median (range)	
Strehlow et al (2016)	Retrospective	Osteopontin	CSF	PCNSL +SCNSL	37	620 ng/ml (60–890 ng/ml)	Inflammatory CNS disease	6	356 ng/ml (253–531 ng/ml)	P < 0.05
			Serum				52 ng/ml (17–231 ng/ml)	Multiple sclerosis Glioblastoma Healthy controls Inflammatory CNS disease Multiple sclerosis Glioblastoma Healthy controls	8 9 13 6 8 9 13	163 ng/ml (29–370 ng/ml) 41 ng/ml (22–191 ng/ml) 319 ng/ml (142–430 ng/ml) 99 ng/ml (40–273 ng/ml)
Kuusisto et al (2015)	Retrospective	AT III	CSF	PCNSL + SCNSL + systemic lymphoma	41	1.47 µg/ml (0.49–6.91 µg/ml)	Alzheimer's (19), Multiple Sclerosis (6), no neurological disease (17)	42	1.08 µg/ml (0.50–4.05 µg/ml)	P = 0.002
			CSF	PCNSL + SCNSL	19	28 pg/ml (≤2–4100 pg/ml) 10.8 pg/ml (1.2–127 pg/ml) 225 U/ml (≤54.5–2750 U/ml)	Glioblastoma (5), anaplastic astrocytoma (2), ependymoma (1), glioma (1), metastasis (5), immature teratoma (1), atypical meningioma (1), undifferentiated sarcoma (1), other (9)	26	<2.0 pg/ml 3.9 pg/ml (0.4–1790 pg/ml) <54.5 U/ml (≤54.5–152 U/ml) 1.5 mg/l (0.3–6.6 mg/l)	
Viaccoz et al (2015)	Retrospective	Neopterin	CSF	PCNSL	28	41.8 nmol/l (7.70–135.0 nmol/l)	Glioma (36), metastasis (13), other type of brain tumour (5) Pseudotumoural inflammatory brain lesion	54	5.1 nmol/l (1.90–16.90 nmol/l) 4.3 nmol/l (1.9–29.2 nmol/l)	PCNSL vs. tumoural and pseudotumoural groups: P < 0.001
			Serum				3.8 nmol/l (1.7–26.5 nmol/l)	Non-tumefactive inflammatory CNS disorders	29	3.8 nmol/l (1.7–26.5 nmol/l)
Kitai et al (2013)	Retrospective	sIL-2R	Serum	PCNSL	13	629.5 ± 586.0 U/ml	Other CNS malignancy (14), CNS inflammation (3), other (7)	24	408.5 ± 250.7 U/ml	NS
			Serum	PCNSL†	12	646.0 ± 609.1 U/ml	Other CNS malignancy (11), CNS inflammation (3), other (5)†	19	347.0 ± 166.6 U/ml	P < 0.025

Table III. (Continued)

Study	Retro- or prospective	Marker	Serum or CSF	Patient group			Control group			P-value
				Diagnosis	N	Mean ± SD or median (range)	Diagnosis (n)	N	Mean ± SD or median (range)	
Rubenstein <i>et al</i> (2013)	Retrospective	CXCL13	CSF	PCNSL new diagnosis	43	5926.2 ± 2030 pg/ml	Neuro-inflammation	71	44.9 ± 19 pg/ml	Recurrent CNS lymphoma vs. new diagnosis: $P < 0.0005$ new diagnosis CNS lymphoma vs. all controls: $P < 1 \times 10^{-7}$ All CNS lymphoma vs. all controls $P < 2.3 \times 10^{-5}$ NS
		CXCL13	CSF	SCNSL recurrent	10	1783 ± 896 pg/ml	Primary brain tumour	8	84.5 ± 60.7 pg/ml	
		CXCL13	CSF	PCNSL recurrent	17	996 ± 312 pg/ml	Brain metastasis	12	58.46 ± 41.6 pg/ml	
		CXCL13	CSF	SCNSL recurrent	13	539 ± 157 pg/ml	Neoplasm/infection outside CNS	46	11 ± 3.6 pg/ml	
Baraniskin <i>et al</i> (2012a)	Retrospective	miR-21	Serum	PCNSL	14	22.60 ± 0.12 Ct values	Other	8	22.63 ± 0.58 Ct values	
	Retrospective	IL-10	CSF	PCNSL new diagnosis	43	282.9 ± 113 pg/ml	Neuro-inflammation	71	5.6 ± 2.3 pg/ml	
		IL-10	CSF	SCNSL new diagnosis	10	57 ± 37 pg/ml	Primary brain tumour	8	10.9 ± 5.6 pg/ml	
		IL-10	CSF	PCNSL recurrent	17	1663 ± 483 pg/ml	Brain metastasis	12	5.3 ± 1.5 pg/ml	
Sasayama <i>et al</i> (2012)	Retrospective	IL-10	CSF	SCNSL recurrent	13	302 ± 126 pg/ml	Neoplasm/infection outside CNS	46	3.6 ± 1.4 pg/ml	
		IL-10	CSF	PCNSL	14	22.60 ± 0.12 Ct values	Other	8	22.63 ± 0.58 Ct values	
		IL-10	CSF	PCNSL	26	27 pg/ml (<2-1610 pg/ml)	Other CNS malignancy	40	All <2.0 pg/ml	$P < 0.001$
		IL-6	CSF	PCNSL	26	5.7 pg/ml (1.2-264 pg/ml)	Other CNS malignancy	40	2.7 pg/ml (0.8-478 pg/ml)	$P = 0.017$
Fischer <i>et al</i> (2009)	Retrospective	B2M	CSF	PCNSL	26	4084 µg/l (970-11239 µg/l)	Other CNS malignancy	40	1200 µg/l (172-2600 µg/l)	$P < 0.001$
		sIL-2R	CSF	PCNSL	26	100 U/ml (<50-978 U/ml)	Other CNS malignancy	40	<50 U/ml (<50-87 U/l)	$P < 0.001$
		IL-10	CSF	PCNSL	5	42 pg/ml (5-118 pg/ml)	Other CNS malignancy (17), CNS inflammation	19	<2.0 pg/ml (2.0-9.0 pg/ml)	$P < 0.001$
		IL-6	CSF	PCNSL	5	6.8 pg/ml (4.4-13.9 pg/ml)	Other CNS malignancy (17), CNS inflammation	19	2.7 pg/ml (0.8-221 pg/ml)	$P = 0.017$
Baraniskin <i>et al</i> (2011)	Retrospective	B2M	CSF	PCNSL	5	4983 µg/l (2303-6166 µg/l)	Other CNS malignancy (17), CNS inflammation	19	1164 µg/l (410-2075 µg/l)	$P < 0.001$
		sIL-2R	CSF	PCNSL	5	88 U/ml (<50-3495 U/ml)	Other CNS malignancy (17), CNS inflammation	19	All <50 U/ml	$P < 0.001$
		miR-21	CSF	PCNSL	23	23.5 REL (7.9-291.7 REL)	CNS inflammation (20), other (10)	30	1.65 REL (0-23.8 REL)	
		miR-19b	CSF	PCNSL	23	6.8 REL (1.4-94.9 REL)	CNS inflammation (20), other (10)	30	0.25 REL (0-5.1 REL)	
Kara <i>et al</i> (2007)	Retrospective	sCD27	CSF	CNS localization of ALL, AML, NHL	5	141.5 pg/ml (31.43-459.60 pg/ml)	Brain metastasis ALL (15), NHL (6), AML (4), epidural hemorrhage (2), lumbar disc protrusion (3)	13	2.6 µg/l§	$P = 0.0005$ $P < 0.0001$
		ATIII	CSF	PCNSL and SCNSL	24	1.68 µg/l§	Malignancy outside CNS (9), other (20)‡ Malignancy outside CNS (29), CNS inflammation (15), other (33)	77	0.54 µg/l§	$P < 3.3 \times 10^{-9}$ , when normalized for total CSF protein $P < 0.015$

Table III. (Continued)

Study	Patient group			Control group			P-value			
	Retro- or prospective	Marker	Serum or CSF	Diagnosis	N	Mean $\pm$ SD or median (range)				
Salmaggi <i>et al</i> (2000)	Retrospective	IL-10	CSF	PCNSL	11	14.8 $\pm$ 23 pg/ml	Other CNS malignancy	11	0.23 $\pm$ 0.26 pg/ml	$P < 0.01$
Murase <i>et al</i> (1998)	Retrospective	sCD27	CSF	PCNSL	12	84.5 $\pm$ 133 U/ml	Other CNS malignancy	30	4.6 $\pm$ 3.2 U/ml	$P < 0.001$
Emerudh <i>et al</i> (1987)	Retrospective	B2M	CSF	SCNSL	13	3.9 $\pm$ 3.4 mg/l	NHL without CNS involvement	11	1.7 $\pm$ 1.0 mg/l	$P < 0.05$
Mavlight <i>et al</i> (1980)	Retrospective	B2M	CSF	SCNSL	6	4.2 $\pm$ 0.6 mg/l	NHL without CNS involvement	14	1.9 $\pm$ 0.3 mg/l	$P < 0.02$

PCNSL, primary central nervous system lymphoma; SCNSL, secondary central nervous system lymphoma; NHL, non-Hodgkin lymphoma; IQR, interquartile range; SD, standard deviation; Ct, cycle threshold; REL, expression of miRNA relative to miR-24; NS, no significant difference.

\*Only values given for subgroups of PCNSL patients, not PCNSL versus control.

†Patients with CRP levels  $>1$  mg/ml excluded.

‡These data are based on a slightly larger group than the group with available CSF data.

§Mean value, no SD reported.

the CXCL13 levels in CSF of CNS lymphoma patients compared with both control groups. The largest study on diagnostic markers for CNS lymphoma included in this review ( $n = 220$ , including relevant disease controls) showed a sensitivity of 69.9% and a specificity of 92.7% for an increased CSF CXCL13 level above 90 pg/ml for CNS lymphoma (Rubenstein *et al*, 2013). The combination of elevated CXCL13 ( $>116$  pg/ml) was combined elevated IL-10 ( $>23$  pg/ml) in the diagnostic set that only applied to the patient group in which the diagnostic question is most relevant, i.e. the patients with non-human immunodeficiency virus (HIV)-associated PCNSL, the diagnostic value of these markers was even better. The sensitivity of the elevation of either CXCL13 or IL-10 above these cut-offs for the latter patient group was 84.2% and the specificity of elevation of both markers was 99.3%. A third study examined the combined diagnostic performance of CSF CXCL-13, CSF IL-10 and the apparent diffusion coefficient (ADC) on brain magnetic resonance imaging (MRI) (Mabray *et al*, 2016). In a cohort of 38 PCNSL and five SCNSL patients, the authors reported a sensitivity of 76.7% and a specificity of 90.9% of CSF CXCL-13 at a cut-off value of  $>103$  pg/ml, when compared to 44 control patients with other brain tumours or tumefactive demyelinating lesions. Combination of CSF CXCL-13, CSF IL-10 and average ADC was statistically significantly superior to the single variables in this patient group, with an area under the receiver-operator characteristic (ROC) curve of 0.928.

#### Interleukin-6

The diagnostic value of CSF IL-6, a lymphoid growth and differentiation cytokine, at a cut-off of 4.0 pg/ml resulted in a sensitivity of 77% and a specificity of 63% (Sasayama *et al*, 2012). Another study on B cell PCNSL patients found a statistically significant difference of CSF IL-6, when compared to patients with non-Hodgkin lymphoma (NHL) involving the CNS and reported low sensitivity and specificity (Song *et al*, 2016). Ranges of IL-6 levels in patient and control groups overlapped to a large extent in both studies. Two studies analysed the diagnostic value of the IL-10/IL-6 ratio. Sasagawa *et al* (2015) found a sensitivity and specificity of 68.4% and 96.1% respectively with the optimal cut-off value of 2.2, while Song *et al* (2016) reported a much higher sensitivity of 95.6% and a specificity of 100%. The latter was the larger of the two studies ( $n = 102$ ) and used a cut-off value of 0.72 (Song *et al*, 2016).

#### Interleukin-8

IL-8, a pro-inflammatory cytokine produced by several cell types, was examined by one study on B cell PCNSL, which reported low sensitivity of 31.8% and specificity of 83.1% at a cut-off value of CSF IL-8 of 117 pg/ml (Song *et al*, 2016).

### Interleukin-10

Six different studies examined the diagnostic value of CSF IL-10, an anti-inflammatory cytokine, in the diagnosis of CNS lymphoma (Sasayama *et al*, 2012; Rubenstein *et al*, 2013; Sasagawa *et al*, 2015; Mabray *et al*, 2016; Nguyen-Them *et al*, 2016; Song *et al*, 2016). The cut-off values for CSF IL-10 that delivered the highest accuracy varied strongly among the studies, between 3.0 and 21.77 pg/ml. Two other studies on IL-10 did not report sensitivity and specificity, but did report a significant difference in the CSF IL-10 concentrations between CNSL patients and controls (Ikeguchi *et al*, 2017; Salmaggi *et al*, 2000).

### Soluble interleukin-2 receptor

Interleukin-2 plays a role in T cell activation and in the maintenance of homeostatic immune responses that prevent autoimmunity (Gaffen, 2001). Its receptor is mainly expressed on activated T cells and is also partially released in the environment as sIL-2R. Four studies on sIL-2R showed diverging results on the utility of this marker in diagnosing CNS lymphoma. First, a sensitivity of 81% was found for increased sIL-2R in CSF, with a cut-off of 77 u/ml, for correct diagnosis of patients initially suspected of having a PCNSL, but specificity was only 56.7% (Sasayama *et al*, 2012). A second study measured sIL-2R values in serum. The difference in mean sIL-2R concentration between the PCNSL group and the control group reached significance when patients with high C-reactive protein values were excluded as a means to rule out the effect of general inflammation, but there was still a large overlap between groups, leading the authors to conclude that serum sIL-2R levels are not a sensitive and specific marker for the diagnosis of PCNSL (Kitai *et al*, 2013). The third study measured CSF levels and used a cut-off value of 60.4 u/ml, resulting in a much higher sensitivity of 94.7% with a specificity of 84.6% (Sasagawa *et al*, 2015). Highest specificity (90%) of CSF IL-2R was found by the fourth study, with a sensitivity of 83.3%, but no cut-off value was reported (Ikeguchi *et al*, 2017).

### Soluble CD19

Muñiz *et al* (2014) evaluated the diagnostic value for the diagnosis of SCNSL of the concentration of sCD19 in CSF of patients with DLBCL or Burkitt lymphoma outside the CNS. CD19 is an antigen normally expressed on B-lymphocytes. They found an association between elevation of sCD19 in CSF and the presence of CNS involvement of the lymphoma in 40 patients, wherein the reference test consisted of positive CSF flow cytometry and/or highly suspicious neurological symptoms. For the cut-off value with the highest accuracy, 1.18 ng/ml, sensitivity and specificity in detecting CNS disease in this patient group were 80% and 95% respectively.

### Soluble CD27

CD27 is a member of the tumour necrosis factor family and plays a role in regulating B cell activation and immunoglobulin synthesis. Kersten *et al* (1996) found a good diagnostic value of elevated sCD27 above 10 u/ml in CSF for meningeal involvement in acute lymphatic leukaemia (ALL) and NHL patients. In four PCNSL patients, seven out of eight CSF samples had elevated sCD27 levels (cut-off 10 u/ml), the only negative sample was obtained after dexamethasone treatment and radiotherapy. In the control group, consisting of patients with other CNS malignancies and non-lymphoid malignancies outside the CNS, all but one sample was negative. A different – methodologically flawed – study analysed the diagnostic value of detecting leptomeningeal involvement in patients with ALL, acute myeloid leukaemia and NHL (Kara *et al*, 2007). With a CSF cut-off value of 350 u/ml, no significant correlation was found. Murase *et al* (2000) investigated the utility of sCD27 levels in the diagnosis of PCNSL and demonstrated a large difference in CSF sCD27 in between 12 PCNSL patients and 30 control patients with other brain tumours. With a cut-off of 15 u/ml, all patients were classified correctly. There was great variability of cut-off levels between studies.

### Tumour necrosis factor- $\alpha$

The pro-inflammatory cytokine TNF- $\alpha$  plays a central role in immune responses and it is suggested that serum levels are correlated with prognosis in DLBCL patients (Lech-Maranda *et al*, 2010). For this reason, Song *et al* (2016) examined the diagnostic value of CSF TNF- $\alpha$  in B cell PCNSL patients, but found low sensitivity and specificity of 59.1% and 57.1% respectively, with a cut-off value of 5.3 pg/ml.

### Beta-2-microglobulin

Several studies have focused on the potential of B2M, a component of major histocompatibility complex class I molecules, as a marker of CNS involvement in lymphoid malignancies. Only studies that reported separate data for primary or secondary CNS lymphoma are presented in Table I. The best accuracy for the diagnosis PCNSL was obtained with a CSF B2M cut-off value of 2.06 mg/l, with an AUC of 0.934 (Sasayama *et al*, 2012). A slightly higher cut-off value of 2.4 mg/l in CSF was used to differentiate CNS lymphoma from non-lymphomas with a sensitivity and specificity of 89.4% and 88.5% respectively (Sasagawa *et al*, 2015). A CSF cut-off value of 2.56 mg/l was found to have the highest accuracy in detecting CNS involvement in patients with DLBCL and Burkitt lymphoma (Muñiz *et al*, 2014). Further, a small study showed a significant difference in B2M CSF levels between NHL patients with and without CNS involvement (Mavlight *et al*, 1980). In a slightly larger study, this difference in CSF B2M levels between NHL

patients with and without CNS involvement was also present, but there was a large overlap between the two groups ( $3.9 \pm 3.5$  mg/l and  $1.7 \pm 1.0$  mg/l for patients with and without CNS involvement of NHL, respectively). Specificity and sensitivity derived from these data were only 71% and 67% respectively for a cut-off of 1.9 mg/l (Ernerudh *et al*, 1987). Finally, the diagnostic value for B2M was also examined in a group of NHL and ALL patients with suspected CNS involvement (Kersten *et al*, 1996). The authors found that the CSF/serum ratio provided a slightly better discrimination between the presence and absence of CNS involvement than CSF levels of B2M alone. However, they also reported that the B2M ratio was also increased in 9 of 40 samples from 32 patients with other CNS malignancy or non-lymphoid malignancy without CNS involvement.

### Anti-thrombin III

Roy *et al* (2008) identified 80 proteins in the CSF of 24 patients with CNS lymphoma compared to 77 control patients without CNS malignancy and evaluated the diagnostic value of one of these markers, antithrombin III (ATIII). ATIII is an enzyme that inactivates thrombin (coagulation factor IIa). Elevation of ATIII above 1.2 µg/ml was 75% sensitive and 98.7% specific in this study population. When normalized for total CSF protein, the ATIII concentrations in patients with CNS lymphoma were still significantly higher than in control patients ( $P < 0.015$ ). However, the mean CSF value of ATIII in 13 patients with CNS metastasis of breast and lung cancer was also elevated (2.6 µg/ml vs. 1.68 µg/ml in CNS lymphoma patients).

A statistically significant difference in median CSF ATIII concentration was also found in another group of lymphoma patients compared to non-neoplastic neurological disorders ( $P = 0.002$ ), and PCNSL patients on the one hand compared to SCNSL or patients without CNS lymphoma lesions on the other ( $P = 0.006$ ) (Kuusisto *et al*, 2015). However, the distribution of ATIII concentrations overlap between the different groups, indicating a poor discriminative value of ATIII.

### sTACI and sBCMA

Transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA) are receptors involved in the B cell homeostasis system. It was recently demonstrated that both receptors also exist in a soluble form (Thaler *et al*, 2017). A recent study on PCNSL patients described superior diagnostic value of sTACI over sBCMA in CSF (Thaler *et al*, 2017). The highest specificity (96.7%) was reported for the combination of sTACI and sBCMA, with cut-off values of 68.4 and 460 pg/ml respectively, and with a sensitivity of 63.9%.

### Neopterin

Neopterin is a non-specific marker of the type 1 T-helper cell derived immune response. It is produced by human monocytes/macrophages upon stimulation with interferon- $\gamma$ . Elevated neopterin levels in serum, CSF and urine have been associated with different infectious and inflammatory diseases, such as HIV infections, but also with auto-immune diseases and malignant tumours (Murr *et al*, 2002). Viacoz *et al* (2015) compared CSF neopterin levels in PCNSL patients with patients with other brain tumours or inflammatory CNS lesions. They found a sensitivity of 96% and a specificity of 92% at a cut-off value of 10 nmol/l.

### Osteopontin

Another pro-inflammatory cytokine, osteopontin, is associated with progression, metastatic spread and poor prognosis in many malignancies (Strehlow *et al*, 2016). In a combined patient group of PCNSL and SCNSL, CSF osteopontin showed a sensitivity of 87% and a specificity of 86%, compared to control patients, with a cut-off value of 400 ng/ml (Strehlow *et al*, 2016). Serum osteopontin demonstrated poor diagnostic value in this population.

### Quality of the studies

The quality of the studies included in this systematic review is presented in Tables IV (per study) and V (per marker). All studies were at risk of bias, due to the risk of (i) bias in patient selection and (ii) bias introduced by post-hoc threshold determination in the index test. Only nine studies reported on a consecutive sample of patients (Kersten *et al*, 1996; Baraniskin *et al*, 2011, 2016; Kitai *et al*, 2013; Muñiz *et al*, 2014; Mabray *et al*, 2016; Nguyen-Them *et al*, 2016; Ikeguchi *et al*, 2017; Thaler *et al*, 2017), therefore the remaining studies were at high or unclear risk of bias on this item. The study by Baraniskin *et al* (2012a) did not report how additional patients [above those already included in Baraniskin *et al* (2011)] were included, so the risk of bias in this study was scored as unclear. Kara *et al* (2007) reported a patient sample of seven NHL patients, of whom only one had leptomeningeal involvement. The majority of patients was diagnosed with leukaemia, which did not correspond to the research question. Concerning the index test, only five studies (Ernerudh *et al*, 1987; Baraniskin *et al*, 2012a; Kara *et al*, 2007; Sasagawa *et al*, 2015; Viacoz *et al*, 2015) used pre-specified cut-off values in determining diagnostic value and seven studies (Mavligit *et al*, 1980; Salmaggi *et al*, 2000; Fischer *et al*, 2009; Kitai *et al*, 2013; Kuusisto *et al*, 2015; Baraniskin *et al*, 2016; Ikeguchi *et al*, 2017) did not use a threshold, but compared mean levels of the marker in patient and control group. Hence, risk of bias concerning the index test was high in the other thirteen studies. There was no reference standard described in six studies (Murase *et al*, 2000; Kara *et al*, 2007; Roy *et al*, 2008; Rubenstein *et al*,



Table IV. Quality of the included studies.

Study	Risk of Bias				Concerns regarding applicability		
	Patient selection	Index tests	Reference standard	Flow and timing	Patient selection	Index tests	Reference standard
Ikeguchi <i>et al</i> (2017)	●	●	●	●	●	●	●
Thaler <i>et al</i> (2017)	●	●	●	●	●	●	●
Baraniskin <i>et al</i> (2016)	●	●	●	●	●	●	●
Mabray <i>et al</i> (2016)	●	●	●	●	●	●	●
Nguyen-Them <i>et al</i> (2016)	●	●	●	●	●	●	●
Song <i>et al</i> (2016)	●	●	●	●	●	●	●
Strehlow <i>et al</i> (2016)	●	●	●	●	●	●	●
Kuusisto <i>et al</i> (2015)	●	●	●	●	●	●	●
Sasagawa <i>et al</i> (2015)	●	●	●	●	●	●	●
Viaccoz <i>et al</i> (2015)	●	●	●	●	●	●	●
Mao <i>et al</i> (2014)	●	●	●	●	●	●	●
Muñiz <i>et al</i> (2014)	●	●	●	●	●	●	●
Kitai <i>et al</i> (2013)	●	●	●	●	●	●	●
Rubenstein <i>et al</i> (2013)	●	●	●	●	●	●	●
Baraniskin <i>et al</i> (2012a)	●	●	●	●	●	●	●
Sasayama <i>et al</i> (2012)	●	●	●	●	●	●	●
Baraniskin <i>et al</i> (2011)	●	●	●	●	●	●	●
Fischer <i>et al</i> (2009)	●	●	●	●	●	●	●
Roy <i>et al</i> (2008)	●	●	●	●	●	●	●
Kara <i>et al</i> (2007)	●	●	●	●	●	●	●
Salmaggi <i>et al</i> (2000)	●	●	●	●	●	●	●
Murase <i>et al</i> (1998)	●	●	●	●	●	●	●
Kersten <i>et al</i> (1996)	●	●	●	●	●	●	●
Ernerudh <i>et al</i> (1987)	●	●	●	●	●	●	●
Mavlight <i>et al</i> (1980)	●	●	●	●	●	●	●

● low risk of bias; ● unclear risk of bias; ● high risk of bias.  
 [Colour figure can be viewed at wileyonlinelibrary.com]

2013; Mao *et al*, 2014; Strehlow *et al*, 2016) and there was possible risk of bias in the way the used reference standard classified the presence or absence of CNS lymphoma in three studies (Ernerudh *et al*, 1987; Mavlight *et al*, 1980; Muñiz *et al*, 2014).

There were concerns regarding the applicability of data from 15 of the reviewed articles (Murase *et al*, 2000; Salmaggi *et al*, 2000; Kara *et al*, 2007; Roy *et al*, 2008; Fischer *et al*, 2009; Baraniskin *et al*, 2011, 2012a, 2016; Rubenstein *et al*, 2013; Mao *et al*, 2014; Kuusisto *et al*, 2015; Song *et al*, 2016; Strehlow *et al*, 2016; Ikeguchi *et al*, 2017; Thaler *et al*, 2017). These concerns resulted from the composition of the control groups, which either contained irrelevant diagnoses or lacked important differential diagnoses ('lymphoma mimics'). Control groups either consisted of healthy subjects (Mao *et al*, 2014; Strehlow *et al*, 2016), patients with non-neurological disease (Kuusisto *et al*, 2015) or neurological disease that are usually not included in the differential diagnosis of PCNSL (e.g. tension-type headache, Alzheimer) (Baraniskin *et al*, 2011, 2012a, 2016), or lacked patients with CNS malignancies (Roy *et al*, 2008; Fischer *et al*, 2009) or CNS inflammation (Murase *et al*, 2000; Salmaggi *et al*, 2000). Rubenstein *et al*

(2013) analysed patients with SCNSL and PCNSL as one group. However, in clinical practice the diagnostic question as well as the differential diagnosis for these conditions is different, resulting in concerns regarding the applicability of the patient selection in this study as well as five other studies (Roy *et al*, 2008; Fischer *et al*, 2009; Mabray *et al*, 2016; Strehlow *et al*, 2016; Ikeguchi *et al*, 2017).

### Discussion

In this systematic review, we searched for markers in CSF or serum with potential value for the diagnosis of CNS lymphoma. We found evidence for the diagnostic utility of the following markers: miR-21, miR-19b, miR-92a, RNU2-1f, CXCL-13, IL-6, IL-8, IL-10, sIL-2R, sCD19, sCD27, TNF- $\alpha$ , B2M, ATIII, sTACI, sBCMA, neopterin and osteopontin in CSF, and for miR-21, sCD27, and B2M in blood.

#### Optimal clinical value

In the interpretation of studies of diagnostic test accuracy, the specific clinical situation dictates what the optimal



Table V. Quality assessment of data, sorted per biomarker.

Marker	No. of studies	CSF/Serum	Risk of bias				Concerns regarding applicability		
			Patient selection	Index tests	Reference standard	Flow and timing	Patient selection	Index tests	Reference standard
miR-21	1	Serum	●	●	●	●	●	●	●
	4	CSF	●	●	●	●	●	●	●
miR-19b	2	CSF	●	●	●	●	●	●	●
miR-92a	2	CSF	●	●	●	●	●	●	●
RNU2-1f	1	CSF + Serum	●	●	●	●	●	●	●
CXCL13	3	CSF	●	●	●	●	●	●	●
IL-6	3	CSF	●	●	●	●	●	●	●
IL-8	1	CSF	●	●	●	●	●	●	●
IL-10	8	CSF	●	●	●	●	●	●	●
IL-10/IL-6	2	CSF	●	●	●	●	●	●	●
sIL-2R	3	CSF	●	●	●	●	●	●	●
	1	Serum	●	●	●	●	●	●	●
sCD19	1	CSF	●	●	●	●	●	●	●
sCD27	3	CSF	●	●	●	●	●	●	●
TNF- $\alpha$	1	CSF	●	●	●	●	●	●	●
B2M	6	CSF	●	●	●	●	●	●	●
AT III	2	CSF	●	●	●	●	●	●	●
sTACI	1	CSF	●	●	●	●	●	●	●
sBCMA	1	CSF	●	●	●	●	●	●	●
Neopterin	1	CSF	●	●	●	●	●	●	●
Osteopontin	1	CSF	●	●	●	●	●	●	●

miR, micro-RNA; RNU, small nuclear RNA; CXCL, chemokine ligand; IL, interleukin; s-IL-R, soluble interleukin receptor; sCD, soluble CD; B2M, beta-2-microglobulin; AT, anti-thrombin; sTACI, soluble transmembrane activator and calcium modulator and cyclophilin ligand interactor; sBCMA, B cell maturation antigen.

● low risk of bias; ● unclear risk of bias; ● high risk of bias.

[Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

diagnostic yield would be. For a certain diagnosis of CNS lymphoma, specifically PCNSL, high specificity is more important than sensitivity, to avoid false-positive diagnoses. Conversely, as a screening tool that should detect all possible cases of (P)CNSL, high sensitivity is most important. In current practice, clinical and radiological features already raise suspicion on CNS lymphoma. A test that could replace the need for biopsy to confirm the diagnosis of CNS lymphoma should ideally have no false positive results, so that the diagnosis of CNS lymphoma is certain and treatment can be initiated. High specificity is therefore the important test quality. A specificity of  $\geq 90\%$  – representing the potential to be developed into a replacement for current reference tests – was reported for combined sTACI and sBCMA (Thaler *et al*, 2017), RNU2-1f (Baraniskin *et al*, 2016), miR-21 (Baraniskin *et al*, 2016), the diagnostic tree of miR-21, miR-19b and miR-92a (Baraniskin *et al*, 2011), neopterin (Viacoz *et al*, 2015), sCD19 (Muñiz *et al*, 2014), sCD27 (Kersten *et al*, 1996; Murase *et al*, 2000), CXCL-13 (Rubenstein *et al*, 2013; Mabray *et al*, 2016), IL-10 (Sasayama *et al*, 2012; Rubenstein *et al*, 2013; Sasagawa *et al*, 2015; Mabray *et al*, 2016; Song *et al*, 2016), IL-10/IL6 ratio (Sasagawa *et al*, 2015; Song *et al*, 2016), B2M (Sasayama *et al*, 2012; Muñiz *et al*, 2014), and

AT-III (Roy *et al*, 2008). Muñiz *et al* (2014) however only studied patients with SCNSL and in several of the available studies (Murase *et al*, 2000; Roy *et al*, 2008; Rubenstein *et al*, 2013; Baraniskin *et al*, 2016; Song *et al*, 2016; Thaler *et al*, 2017), concerns regarding applicability data were high because of patient selection issues.

#### Considerations on individual markers

MicroRNAs (miR) and small nuclear RNAs (snRNA) play key roles in many (haematological) cellular pathways and dysfunction of microRNAs is found to be a feature of malignancies (Calin & Croce, 2006; Lawrie, 2007). Mao *et al* (2014) reported a large area under the ROC-curve for levels of miR-21 in serum; Baraniskin *et al* (2012a), however, reported no elevation of miR-21 in serum compared to controls. This might be due to the different composition of the control groups. In Mao *et al* (2014) the control group contained healthy people, which does not reflect clinical practice. Since miR-21 was also found to be elevated in the serum of patients with glioblastoma (Baraniskin *et al*, 2012b), future studies on miR-21 in CSF should compare PCNSL patients to adequate disease controls including glioblastoma patients.

Mao *et al* (2014) did report diagnostic value for miR-21 in serum when CNS lymphoma patients were compared to patients with glioblastoma.

Rubenstein *et al* (2013) found good diagnostic accuracy for CXCL13 in CSF in a large-scale study. Importantly, this marker was also elevated in half of the patients with brain metastases of breast cancer. Clinical use of this marker may lead to incorrect diagnosis of PCNSL in patients with CNS metastases of breast cancer, specifically when there is no known diagnosis of breast cancer. Mabray *et al* (2016) endorses the good diagnostic value of CXCL13 in CSF, especially when it is combined with CSF IL-10 and average ADC on brain MRI. Of note, CXCL13 is often positive in cases of neuroborreliosis, and has in fact been identified as a useful marker to discriminate neuroborreliosis from other neuro-inflammatory diseases (Schmidt *et al*, 2011). Neuroborreliosis, however, is usually not one of the main differential diagnoses for CNS lymphoma.

Individual interleukins were examined in various studies, but none of them reached good diagnostic value, due to wide variation in cut-off value, low sensitivity or low methodological quality of the investigating study. Blood or CSF interleukin levels can also be elevated in other conditions included in the differential diagnosis of CNS lymphoma. CSF IL-10 for example, is found to be elevated in neuro-inflammatory and -infectious diseases (Wang *et al*, 2017). IL-6 secretion is stimulated in Waldenström macroglobulinaemia and increased levels can be detected in the blood of patients with Bing Neel syndrome (Elsawa *et al*, 2011). Nevertheless, the ratio of IL-10 and IL-6 is already used in differentiating intraocular lymphoma (ratio > 1) from uveitis (Kimura *et al*, 2012). Sasagawa *et al* (2015) found high specificity, but relatively low sensitivity, of this ratio in CSF for distinguishing CNS lymphoma from other CNS brain lesions; patients with inflammatory diseases were scarce in the control group. In line with the findings in ocular disease, the IL-10/IL-6 ratio would also be expected to be lower in inflammatory CNS diseases than in CNS lymphoma. However, Sasayama *et al* (2012) found moderate diagnostic value for elevated IL-6 levels  $\geq 4.0$  pg/ml for CNS lymphoma (regardless of IL-10 level); this seemingly counterintuitive finding may be due to the fact that the control group consisted mainly of patients with other CNS malignancies and only contained two patients with CNS inflammation. Song *et al* (2016) did include a significant number of patients with inflammatory CNS diseases in their control group and found high specificity as well as high sensitivity of the IL-10/IL-6 ratio, however the cut-off value (a ratio of 0.72) was not predetermined and differed from previous reports.

Although sCD27 showed good diagnostic value in three studies that compared PCNSL with other CNS disease, these studies suffered from inadequate control groups and small numbers of PCNSL patients (two studies combined PCNSL with other haematological malignancies) (Kersten *et al*, 1996; Murase *et al*, 2000; Kara *et al*, 2007).

sCD19 has only been evaluated as a marker of CNS involvement in systemic DLBCL or BL, compared with patients who had DLBCL or BL without CNS involvement as a control group. The diagnostic value of this marker still needs to be determined for patients with primary CNS lymphoma, with other brain tumours and CNS inflammation as a control group.

B2M has already been identified as a biomarker for a broader spectrum of lymphoid malignancies (Pudek *et al*, 1985; Ernerudh *et al*, 1987; Oberg *et al*, 1987; Mavligit *et al*, 1980). This may explain the difference in diagnostic values found among the studies. Two studies reported good diagnostic value of B2M in CSF when PCNSL patients were compared to patients with other brain malignancies (Sasayama *et al*, 2012; Sasagawa *et al*, 2015). The comparison of SCNSL patients with other lymphoid malignancies without CNS involvement, however, resulted in poor diagnostic values (Ernerudh *et al*, 1987; Kersten *et al*, 1996; Muñoz *et al*, 2014). None of the studies on B2M included a significant number of patients with neuro-inflammation in their control group.

Great differences in concentration of the sIL-2R between PCNSL patients and controls was reported by Sasayama *et al* (2012) when measured in CSF. Moreover, Sasagawa *et al* (2015) and Ikeguchi *et al* (2017) endorse this diagnostic value of CSF sIL-2R, although their patient groups were small and also comprised SCNSL. Kitai *et al* (2013) however showed that sIL-2R was not a good diagnostic marker when measured in serum, due to the elevation of sIL-2R in patients with inflammatory disease. Serum sIL-2R is also a marker of disease activity in patients with sarcoidosis and a criterium in haemophagocytic lymphohistiocytosis, and in CSF it is frequently used as an indicator for neurosarcoidosis (Grutters *et al*, 2003; Peterit *et al*, 2010). There were only a few patients with inflammatory disease in the control groups of Sasayama *et al* (2012), Sasagawa *et al* (2015) and Kitai *et al* (2013). The majority of control patients from Ikeguchi *et al* (2017) had multiple sclerosis, however, the authors did not make it clear which control group was used for calculating the diagnostic value. It should be further investigated whether sIL2R concentration in CSF can help to distinguish between CNS lymphoma and inflammatory disease.

Antithrombin III could not discriminate between CNS lymphoma and cerebral metastasis (Roy *et al*, 2008) or non-neoplastic neurological diseases (Kuusisto *et al*, 2015) and is likely to be a non-specific marker of blood-brain barrier disruption (Zetterberg *et al*, 2009). However, it might be useful in the demonstration of CNS involvement in peripheral NHL (i.e. SCNSL), although this has not yet been validated.

The diagnostic value of sTACI and sBCMA was examined by one study of moderate methodological quality (Thaler *et al*, 2017). sTACI showed superior diagnostic value over sBCMA and combination of the two biomarkers in CSF revealed highest specificity, at the expense of a lower sensitivity.

Viaccoz *et al* (2015) were the first to describe neopterin as a biomarker for PCNSL, as it previously had been used as a marker for several infectious and inflammatory diseases. This low-bias study shows promising diagnostic accuracy of neopterin for the differentiation between PCNSL and other brain tumours, pseudotumoural inflammatory lesions or nontumefactive inflammatory CNS disorders. However, sample sizes were limited and, given that high CSF neopterin levels are reported in demyelinating disease (Viaccoz *et al*, 2015), larger studies are needed, including patients with pseudotumoural infectious and inflammatory disease.

Osteopontin showed good potential as a CSF biomarker for CNSL, but methodological quality of the study was very poor and therefore these results have to be interpreted with caution (Strehlow *et al*, 2016).

Circulating cell-free DNA in blood has been identified as a biomarker for tumour burden and malignant progression in cancer patients (Schwarzenbach *et al*, 2011). Recently, this biomarker was also used to identify somatic mutations of PCNSL in blood or CSF, which has potential for individualizing antineoplastic treatments (Fontanilles *et al*, 2017), and possibly as a diagnostic tool; however, no diagnostic studies have been published thus far.

In addition to the diagnostic value of the above-mentioned biomarkers, some authors also emphasized the possible prognostic value of these markers in CNS lymphoma. Elevated microRNA's were negatively related to prognosis in several studies (Baraniskin *et al*, 2011, 2012a; Mao *et al*, 2014) as well as RNU-21f (Baraniskin *et al*, 2016), TNF- $\alpha$  (Lech-Maranda *et al*, 2010; Dlouhy *et al*, 2017) and sCD19 (Muñiz *et al*, 2014). Evidence for CSF IL-10 was conflicting (Salmaggi *et al*, 2000; Lech-Maranda *et al*, 2010; Mizowaki *et al*, 2015; Nguyen-Them *et al*, 2016; Sasayama *et al*, 2016; Wang *et al*, 2017). The association between IL-6 and sIL-2R levels and prognosis of CNS lymphoma patients is not unambiguously reported (Sasayama *et al*, 2016; Dlouhy *et al*, 2017). More evidence of sufficient quality is needed to evaluate the prognostic value of these markers.

## Limitations

This review is based on mostly retrospective studies; only two studies were prospective and one study was partially prospective. The number of studies about each individual marker were low and most markers were studied in small patient cohorts. We could not perform a formal meta-analysis of diagnostic test accuracy for each marker due to the differences between studies in sample selection (specifically the selection of control groups), in thresholds for each marker and in reporting of outcome measures. Quality assessment of the included studies by means of QUADAS-2 demonstrated that all studies were at risk of bias and there was concern regarding applicability of the selected patients and controls in 15 studies (Murase *et al*, 2000; Salmaggi *et al*, 2000; Kara *et al*, 2007; Roy *et al*, 2008; Fischer *et al*, 2009; Baraniskin

*et al*, 2011, 2012a, 2016; Rubenstein *et al*, 2013; Mao *et al*, 2014; Kuusisto *et al*, 2015; Song *et al*, 2016; Strehlow *et al*, 2016; Ikeguchi *et al*, 2017; Thaler *et al*, 2017). Due to these findings, it cannot be determined yet whether the reported diagnostic values of the markers will also hold true in clinical practice. Although some studies reported on the combination of different biomarkers (Baraniskin *et al*, 2011, 2012a; Rubenstein *et al*, 2013; Sasagawa *et al*, 2015; Mabray *et al*, 2016; Song *et al*, 2016; Thaler *et al*, 2017), data on the combined use of several of these markers is limited. It is suggested that biomarker profiles of patients, based on several different biomarkers, could show better diagnostic accuracy than single markers (Thijs *et al*, 2017).

## Conclusion

Available evidence on diagnostic markers for the diagnosis of CNS lymphoma in blood or CSF is of limited quality. Methodological issues originate from mostly retrospective small-scale studies with heterogeneous sample selection and test definitions, leading to high risk of bias for all markers but one.

miR-21, miR-19b, miR-92a, RNU2-1f, CXCL-13, IL-10, sIL-2R, sCD19, sCD27, B2M, cTACI and sBCMA, neopterin and osteopontin in CSF and miR-21, and the sIL-2R in blood are possible diagnostic markers for both primary and secondary CNS lymphoma. ATIII has limited utility due to the poor ability to discriminate PCNSL from other malignancies. CXCL-13, B2M and neopterin in CSF show the highest potential to form a diagnostic marker for CNS lymphoma. However, we do not yet recommend application of these markers in clinical practice because of the current insufficient level of empirical evidence. As of now, brain biopsy still remains the gold standard.

We do, however, emphasize the potential of this relatively non-invasive diagnostic tool. The diagnostic value of these markers should be further studied in prospective cohort studies with sufficient numbers of consecutively collected patients, adequate control groups and predefined test thresholds, before they can be used in clinical practice. Moreover, validation of these markers in consecutive cohorts, with simultaneous measurement of several or all of the identified candidate-markers is recommended. When diagnostic value of these markers, or clusters of several markers, is confirmed, stereotactic biopsy might not be longer necessary in some patients or may be hastened in other patients. These changes could lead to faster diagnosis, fewer cases of biopsy-related morbidity and, possibly, better survival and quality of life.

## Authors contributions

All authors contributed to analysis and writing of the manuscript and approved its final version. LCAS and TJS designed the study. LCAS and AW acquired data. LCAS, AW and TJS first analysed the data.

## Funding

No funding was received for this research.

## Competing interests

The authors have no competing interests.

## References

Ansel, K.M., Harris, R.B.S. & Cyster, J.G. (2002) CXCL13 is required for B1 cell homing, natural antibody production, and body cavity immunity. *Immunity*, **16**, 67–76.

Baraniskin, A., Kuhnenn, J., Schlegel, U., Chan, A., Deckert, M., Gold, R., Maghnouj, A., Zöllner, H., Reinacher-Schick, A., Schmiegel, W., Hahn, S.A. & Schroers, R. (2011) Identification of microRNAs in the cerebrospinal fluid as marker for primary diffuse large B-cell lymphoma of the central nervous system. *Blood*, **117**, 3140–3146.

Baraniskin, A., Kuhnenn, J., Schlegel, U., Schmiegel, W., Hahn, S. & Schroers, R. (2012a) MicroRNAs in cerebrospinal fluid as biomarker for disease course monitoring in primary central nervous system lymphoma. *Journal of Neuro-Oncology*, **109**, 239–244.

Baraniskin, A., Kuhnenn, J., Schlegel, U., Maghnouj, A., Zöllner, H., Schmiegel, W., Hahn, S. & Schroers, R. (2012b) Identification of microRNAs in the cerebrospinal fluid as biomarker for the diagnosis of glioma. *Neuro-Oncology*, **14**, 29–33.

Baraniskin, A., Zaslavka, E., Nöpel-Dünnebacke, S., Ahle, G., Seidel, S., Schlegel, U., Schmiegel, W., Hahn, S. & Schroers, R. (2016) Circulating U2 small nuclear RNA fragments as a novel diagnostic biomarker for primary central nervous system lymphoma. *Neuro-Oncology*, **18**, 361–367.

Bellinzona, M., Roser, F., Ostertag, H., Gaab, R.M. & Saini, M. (2005) Surgical removal of primary central nervous system lymphomas (PCNSL) presenting as space occupying lesions: a series of 33 cases. *European Journal of Surgical Oncology*, **31**, 100–105.

Calin, G.A. & Croce, C.M. (2006) MicroRNA-cancer connection: the beginning of a new tale. *Cancer Research*, **66**, 7390–7394.

Dlouhy, I., Filella, X., Rovira, J., Magnano, L., Rivas-Delgado, A., Baumann, T., Martínez-Trillos, A., Balagué, O., Martínez, A., González-Farre, B., Karube, K., Gine, E., Delgado, J., Campo, E. & López-Guillermo, A. (2017) High serum levels of soluble interleukin-2 receptor (sIL-2R), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF) are associated with adverse clinical features and predict poor outcome in diffuse large B-cell lymphoma. *Leukemia Research*, **59**, 20–25.

Dolecek, T.A., Propp, J.M., Stroup, N.E. & Kruchko, C. (2012) CBTRUS statistical report : primary brain and central nervous system

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Details of patient and control groups.

tumors diagnosed in the United States in 2005–2009. *Neuro-Oncology*, **14**, 1–49.

Elsawa, S.F., Novak, A.J., Ziesmer, S.C., Almada, L.L., Hodge, L.S., Grote, D.M., Witzig, T.E., Fernandez-Zapico, M.E. & Ansell, S.M. (2011) Comprehensive analysis of tumor microenvironment cytokines in Waldenstrom macroglobulinemia identifies CCL5 as a novel modulator of IL-6 activity. *Blood*, **118**, 5540–5549.

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

Ferreri, A.J.M. & Marturano, E. (2012) Primary CNS lymphoma. *Best Practice and Research: Clinical Haematology*, **25**, 119–130.

Fischer, L., Korfel, A., Pfeiffer, S., Kiewe, P., Volk, H.D., Cakiroglu, H., Widmann, T. & Thiel, E. (2009) CXCL13 and CXCL12 in central nervous system lymphoma patients. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, **15**, 5968–5973.

Fontanilles, M., Marguet, F., Bohers, É., Viailly, P.J., Dubois, S., Bertrand, P., Camus, V., Mareschal, S., Ruminy, P., Maingonnat, C., Lepretre, S., Verezezan, E.L., Derrey, S., Tilly, H., Picquenot, J.M., Laquerrière, A. & Jardin, F. (2017) Non-invasive detection of somatic mutations using next-generation sequencing in primary central nervous system lymphoma. *Oncotarget*, **8**, 48157–48168.

Gaffen, S.L. (2001) Signaling domains of the interleukin 2 receptor. *Cytokine*, **14**, 63–77.

Grutters, J.C., Fellrath, J.M., Mulder, L., Janssen, R., van den Bosch, J.M. & van Velzen-Blad, H. (2003) Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest*, **124**, 186–195.

Haldorsen, I.S., Espeland, A., Larsen, J.L. & Mella, O. (2005) Diagnostic delay in primary central nervous system lymphoma. *Acta Oncologica (Stockholm, Sweden)*, **44**, 728–734.

Haldorsen, I.S., Espeland, A. & Larsson, E.M. (2011) Central nervous system lymphoma: characteristic findings on traditional and advanced imaging. *American Journal of Neuroradiology*, **32**, 984–992.

Ikeguchi, R., Shimizu, Y., Shimizu, S. & Kitagawa, K. (2017) CSF and clinical data are useful in differentiating CNS inflammatory demyelinating disease from CNS lymphoma. *Multiple Sclerosis Journal*, 2017 Jun 1:1352458517717804. <https://doi.org/10.1177/1352458517717804> [Epub ahead of print].

Kara, I.O., Sahin, B. & Gunesacar, R. (2007) Levels of serum and cerebrospinal fluid soluble CD27 in the diagnosis of leptomeningeal involvement of hematolymphoid malignancies. *Advances in Therapy*, **24**, 741–747.

Kersten, M.J., Evers, L.M., Dellemijn, P.L., van den Berg, H., Portegies, P., Hintzen, R.Q., van Lier, R.A., von dem Borne, A.E. & van Oers, R.H. (1996) Elevation of cerebrospinal fluid soluble CD27 levels in patients with meningeal localization of lymphoid malignancies. *Blood*, **87**, 1985–1989.

Khatab, S., Spliet, W. & Woerdeman, P.A. (2014) Frameless image-guided stereotactic brain biopsies: emphasis on diagnostic yield. *Acta Neurochirurgica*, **156**, 1441–1450.

Kimura, K., Usui, Y. & Goto, H.; Japanese Intraocular Lymphoma Study Group. (2012) Clinical features and diagnostic significance of the intraocular fluid of 217 patients with intraocular lymphoma. *Japanese Journal of Ophthalmology*, **56**, 383–389.

Kitai, R., Sasaki, H., Matsuda, K., Tsunetoshi, K., Yamauchi, T., Neishi, H., Matsumura, K., Tsunoda, A., Takeuchi, H., Sato, K. & Kikuta, K. (2013) Measurement and cellular sources of the soluble interleukin-2 receptor in primary central nervous system lymphoma. *Brain Tumor Pathology*, **30**, 34–39.

Küker, W., Nägele, T., Korfel, A., Heckl, S., Thiel, E., Bamberg, M., Weller, M. & Herrlinger, U. (2005) Primary central nervous system lymphomas (PCNSL): MRI features at presentation in 100 patients. *Journal of Neuro-Oncology*, **72**, 169–177.

Kuusisto, M.E., Haapasari, K.M., Remes, A.M., Bloigu, R., Karihtala, P., Turpeenniemi-Hujanen, T. & Kuittinen, O. (2015) Antithrombin III is probably not a suitable biomarker for diagnosis of primary central nervous system lymphoma. *Annals of Hematology*, **94**, 1167–1174.

Lawrie, C.H. (2007) MicroRNAs and haematology: small molecules, big function. *British Journal of Haematology*, **137**, 503–512.

Lech-Maranda, E., Bienvenu, J. & Broussais-Guillemot, F. (2010) Plasma TNF- $\alpha$  and IL-10 level-based prognostic model predicts outcome of patients with diffuse large B-cell lymphoma in different risk groups defined by the international prognostic index. *Archivum Immunologicae et Therapiae Experimentalis*, **58**, 131–141.

Mabray, M.C., Barajas, R.F., Villanueva-Meyer, J.E., Zhang, C.A., Valles, F.E., Rubenstein, J.L. & Cha, S. (2016) The combined performance of ACD, CSF CXCL-13, and CSF IL-10 in the



- diagnosis of central nervous system lymphoma. *American Journal of Neuroradiology*, **37**, 74–79.
- Mao, X., Sun, Y. & Tang, J. (2014) Serum miR-21 is a diagnostic and prognostic marker of primary central nervous system lymphoma. *Neurological Sciences*, **35**, 233–238.
- Mavligit, G.M., Stuckey, S.E., Cabanillas, F.F., Keating, M.J., Tourtellotte, W.W., Schold, S.C. & Freireich, E.J. (1980) Diagnosis of leukemia or lymphoma in the central nervous system by beta 2-microglobulin determination. *New England Journal of Medicine*, **303**, 718–722.
- Mizowaki, T., Sasayama, T., Tanaka, K., Mizukawa, K., Takata, K., Nakamizo, S., Tanaka, H., Nagashima, H., Nishihara, M., Hirose, T., Itoh, T. & Kohmura, E. (2015) STAT3 activation is associated with cerebrospinal fluid interleukin-10 (IL-10) in primary central nervous system diffuse large B cell lymphoma. *Journal of Neuro-Oncology*, **124**, 165–174.
- Moher, D., Liberati, A., Tetzlaff, J. & Altman, D.G.; PRISMA Group. (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine*, **6**, e1000097.
- Morris, P.G. & Abrey, L.E. (2009) Therapeutic challenges in primary CNS lymphoma. *The Lancet Neurology*, **8**, 581–592.
- Muñiz, C., Martín-Martín, L., López, A., Sánchez-González, B., Salar, A., Almeida, J., Sancho, J.M., Ribera, J.M., Heras, C., Peñalver, F.J., Gómez, M., González-Barca, E., Alonso, N., Navarro, B., Olave, T., Sala, F., Conde, E., Márquez, J.A., Cabezedo, E., Cladera, A., García-Malo, M., Caballero, M.D. & Orfao, A.; Spanish Group for the Study of Central Nervous System Disease in Non-Hodgkin Lymphoma. (2014) Contribution of cerebrospinal fluid sCD19 levels to the detection of CNS lymphoma and its impact on disease outcome. *Blood*, **123**, 1864–1869.
- Murase, S., Saio, M., Takenaka, K., Shinoda, J., Nishimura, Y., Sakai, N. & Takami, T. (1998) Increased levels of CSF soluble CD27 in patients with primary central nervous system lymphoma. *Cancer Letters*, **132**, 181–186.
- Murase, S., Saio, M., Andoh, H., Takenaka, K., Shinoda, J., Nishimura, Y., Sakai, N. & Takami, T. (2000) Diagnostic utility of CSF soluble CD27 for primary central nervous system lymphoma in immunocompetent patients. *Neurological Research*, **22**, 434–442.
- Murr, C., Widner, B., Wirlitner, B. & Fuchs, D. (2002) Neopterin as a marker for immune system activation. *Current Drug Metabolism*, **3**, 175–187.
- Nayak, L., Pentsova, E. & Batchelor, T.T. (2015) Primary CNS lymphoma and neurologic complications of hematologic malignancies. *Continuum (Minneapolis, Minn.)*, **21**, 355–372.
- Nguyen-Them, L., Costopoulos, M., Tanguy, M.L., Houillier, C., Choquet, S., Benanni, H., Elias-Shamieh, R., Armand, M., Faivre, G., Glaisner, S., Malak, S., Vargaftig, J., Hoang-Xuan, K., Ahle, G., Toutou, V., Cassoux, N., Davi, F., Merle-Béral, H., Le Garff-Tavernier, M. & Soussein, C.; French LOC Network for CNS Lymphoma. (2016) The CSF IL-10 concentration is an effective diagnostic marker in immunocompetent primary CNS lymphoma and a potential prognostic biomarker in treatment-responsive patients. *European Journal of Cancer*, **61**, 69–76.
- Oberg, G., Hallgren, R. & Venge, P. (1987) Beta 2-microglobulin, lysozyme and lactoferrin in cerebrospinal fluid in patients with lymphoma or leukaemia: relationship to CNS involvement and the effect of prophylactic intrathecal treatment with methotrexate. *British Journal of Haematology*, **66**, 315–322.
- Petereit, H.F., Reske, D., Tumani, H., Jarius, S., Markus Leweke, F., Woitalla, D., Pfister, H.W. & Rubbert, A. (2010) Soluble CSF interleukin 2 receptor as indicator of neurosarcoidosis. *Journal of Neurology*, **257**, 1855–1863.
- Pudek, M.R., Chan, K.W., Rogers, P.C. & Teasdale, J.M. (1985) Beta2-microglobulin levels in cerebrospinal fluid of children with leukemia and lymphoma. *Clinical Biochemistry*, **18**, 180–183.
- Ricard, D., Idhah, A., Ducray, F., Lahutte, M., Hoang-Xuan, K. & Delattre, J.Y. (2012) Primary brain tumours in adults. *The Lancet*, **379**, 1984–1996.
- Roth, P., Wick, W. & Weller, M. (2010) Steroids in neurooncology: actions, indications, side-effects. *Current Opinion in Neurology*, **23**, 597–602.
- Roy, S., Josephson, S.A., Fridlyand, J., Karch, J., Kadoch, C., Karrim, J., Damon, L., Treseler, P., Kunwar, S., Shuman, M.A., Jones, T., Becker, C.H., Schulman, H. & Rubenstein, J.L. (2008) Protein biomarker identification in the CSF of patients with CNS lymphoma. *Journal of Clinical Oncology*, **26**, 96–105.
- Rubenstein, J.L., Wong, V.S., Kadoch, C., Gao, H.X., Barajas, R., Chen, L., Josephson, S.A., Scott, B., Douglas, V., Maiti, M., Kaplan, L.D., Treseler, P.A., Cha, S., Hwang, J.H., Cinque, P., Cyster, J.G. & Lowell, C. (2013) CXCL13 plus interleukin-10 are highly specific for the diagnosis of CNS lymphoma. *Blood*, **121**, 4740–4749.
- Salmaggi, A., Eoli, M., Corsini, E., Gelati, M., Frigerio, S., Silvani, A. & Boiardi, A. (2000) Cerebrospinal fluid interleukin-10 levels in primary central nervous system lymphoma: a possible marker of response to treatment? *Annals of Neurology*, **47**, 137–138.
- Sasagawa, Y., Akai, T., Tachibana, O. & Iizuka, H. (2015) Diagnostic value of interleukin-10 in cerebrospinal fluid for diffuse large B-cell lymphoma of the central nervous system. *Journal of Neuro-Oncology*, **121**, 177–183.
- Sasayama, T., Nakamizo, S., Nishihara, M., Kawamura, A., Tanaka, H., Mizukawa, K., Miyake, S., Taniguchi, M., Hosoda, K. & Kohmura, E. (2012) Cerebrospinal fluid interleukin-10 is a potentially useful biomarker in immunocompetent primary central nervous system lymphoma (PCNSL). *Neuro-Oncology*, **14**, 368–380.
- Sasayama, T., Tanaka, K., Mizowaki, T., Nagashima, H., Nakamizo, S., Tanaka, H., Nishihara, M., Mizukawa, K., Hirose, T., Itoh, T. & Kohmura, E. (2016) Tumor-associated macrophages associate with cerebrospinal fluid interleukin-10 and survival in primary central nervous system lymphoma (PCNSL). *Brain Pathology (Zurich, Switzerland)*, **26**, 479–487.
- van de Schans, S.A., Issa, D.E., Visser, O., Nooijen, P., Huijgens, P.C., Karim-Kos, H.E., Janssen-Heijnen, M.L. & Coebergh, J.W. (2012) Diverging trends in incidence and mortality, and improved survival of non-Hodgkin's lymphoma, in the Netherlands, 1989–2007. *Annals of Oncology*, **23**, 171–182.
- Schmidt, C., Plate, A., Angele, B., Pfister, H.W., Wick, M., Koedel, U. & Rupperecht, T.A. (2011) A prospective study on the role of CXCL13 in Lyme neuroborreliosis. *Neurology*, **76**, 1051–1058.
- Schwarzenbach, H., Hoon, D.S.B. & Pantel, K. (2011) Cell-free nucleic acids as biomarkers in cancer patients. *Nature Reviews Cancer*, **11**, 426–437.
- Scott, B.J., Douglas, V.C., Tihan, T., Rubenstein, J.L. & Josephson, S.A. (2013) A systematic approach to the diagnosis of suspected central nervous system lymphoma. *JAMA Neurology*, **70**, 311–319.
- Song, Y., Zhang, W., Zhang, L., Wu, W., Zhang, Y., Han, X., Yang, C., Zhang, L. & Zhou, D. (2016) Cerebrospinal fluid IL-10 and IL-10/IL-6 as accurate diagnostic biomarkers for primary central nervous system large B-cell lymphoma. *Scientific Reports*, **6**, 1–8.
- Strehlow, F., Bauer, S., Martus, P., Weller, M., Roth, P., Schlegel, U., Seidel, S., Scheibenbogen, C., Korfel, A. & Kreher, S. (2016) Osteopontin in cerebrospinal fluid as diagnostic biomarker for central nervous system lymphoma. *Journal of Neuro-Oncology*, **129**, 165–171.
- Thaler, F.S., Laurent, S.A., Huber, M., Mulazani, M., Dreyling, M., Ködel, U., Kümpfel, T., Straube, A., Meinel, E. & von Baumgarten, L. (2017) Soluble TACI and soluble BCMA as biomarkers in primary central nervous system lymphoma. *Neuro-Oncology*, **19**, 1618–1627.
- Thijs, J.L., Strickland, I., Buijnzel-Koomen, C.A.F.M., Nierkens, S., Giovannone, B., Csomor, E., Sellman, B.R., Mustelin, T., Sleeman, M.A., de Bruin-Weller, M.S., Herath, A., Drylewicz, J., May, R.D. & Hijnen, D. (2017) Moving towards endotypes in atopic dermatitis: identification of patient clusters based on serum biomarker analysis. *Journal of Allergy and Clinical Immunology*, **140**, 730–737.
- Vafai, P. & DiGiuseppe, J.A. (2014) Detection of B-cell populations with monotypic light chain expression in cerebrospinal fluid specimens from patients with multiple sclerosis by polychromatic flow cytometry. *Cytometry Part B - Clinical Cytometry*, **86**, 106–110.
- Viacoz, A., Ducray, F., Tholance, Y., Barcelos, G.K., Thomas-Maisonneuve, L., Ghesquière,

- H., Meyronet, D., Quadrio, I., Cartalat-Carel, S., Louis-Tisserand, G., Jouanneau, E., Guyotat, J., Honnorat, J. & Perret-Liaudet, A. (2015) CSF neopterin level as a diagnostic marker in primary central nervous system lymphoma. *Neuro-Oncology*, **17**, 1497–1503.
- Wang, L., Luo, L., Gao, Z., Liu, S.F., Liu, C.J., Ma, D.X., Chen, J.G., Cao, W.R., Yin, A.M., Xu, J.W., Wang, S.L., Zhuo, D.J., Geng, B., Zhao, S.S., Wang, F.Y., Yang, N., Guan, L.X., Gu, Z.Y. & Gao, C.J. (2017) The diagnostic and prognostic value of interleukin-10 in cerebrospinal fluid for central nervous system lymphoma: a meta-analysis. *Leukemia & Lymphoma*, **58**, 2452–2459.
- Whiting, P.F., Rutjes, A.W., Westwood, M.E., Mallett, S., Deeks, J.J., Reitsma, J.B., Leeflang, M.M., Sterne, J.A. & Bossuyt, P.M.; QUADAS-2 Group. (2011) Research and reporting methods accuracy studies. *Annals of Internal Medicine*, **155**, 529–536.
- Zacharia, T.T., Law, M., Naidich, T.P. & Leeds, N.E. (2008) Central nervous system lymphoma characterization by diffusion-weighted imaging and MR spectroscopy. *Journal of Neuroimaging*, **18**, 411–417.
- Zetterberg, H., Andreasson, U. & Blennow, K. (2009) CSF antithrombin III and disruption of the blood-brain barrier. *Journal of Clinical Oncology*, **27**, 2302–2303.