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Cerebrospinal fluid biomarkers in bacterial meningitis

Biomarker im Liquor cerebrospinalis bei bakterieller Meningitis

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Abstract

Bacterial meningitis (BM) is a serious infectious disease that results in significant morbidity and mortality. Brain damage is mainly caused by the inflammatory reaction to the invading pathogen. This inflammation leads to the release of biomarkers into the cerebrospinal fluid (CSF). Here we review the literature on CSF biomarkers in BM between 1990 and 2008 and discuss it with regard to its clinical relevance to act as a surrogate for diagnostic purposes, estimation of disease severity as well as prediction of treatment response and clinical outcome. We classify recommendations for these markers as levels A–C according to the scheme approved for the European Federation of Neurological Societies guidelines (EFNS). In conclusion, we identified significantly elevated levels of several inflammatory mediators, acute phase proteins, blood-brain barrier associated proteins and markers for oxidative stress and neuronal damage in the CSF of BM patients compared to aseptic meningitis patients and controls. Furthermore, several biomarkers correlated with disease severity, clinical outcome, and response to antibiotic treatment.

Keywords: bacterial meningitis (BM); biomarker; cerebrospinal fluid (CSF); diagnosis; disease severity; outcome; treatment response.

Zusammenfassung

Die bakterielle Meningitis (BM) ist eine ernstzunehmende Infektionskrankheit, welche eine beträchtliche Morbidität und Mortalität aufweist. Die Schädigung des Gehirns ist hauptsächlich eine Folge der enzündlichen Reaktion gegen das eindringende Pathogen. Diese Entzündung führt zur Freisetzung von Biomarkern in den Liquor cerebrospinalis. In diesem Artikel geben wir einen Überblick über die Evidenz von Liquor Biomarkern bei BM, die zwischen 1990 und 2008 in Hinblick auf deren klinische Relevanz für Diagnostik, Einschätzung des Krankheitsverlaufes und Prädiktion von Therapieansprechen und klinischem Outcome publiziert wurden. Wir klassifizierten die Marker als Level A–C gemäß dem Schema der European Federation of Neurological Societies-Richtlinien. Patienten mit BM zeigten im Liquor cerebrospinalis signifikant erhöhte Konzentrationen von inflammatorischen Mediatoren, Akute-Phase-Proteinen, Bluthirn-Schranken assoziierte Proteinen und Markern für oxidativen Stress und neuronaler Schädigung im Vergleich zu Patienten mit aseptischer Meningitis und Kontrollindividuen. Einige Biomarker korrelierten mit der Schwere der Erkrankung, klinischem Outcome und Ansprechen auf antibiotische Therapie.

Schlüsselwörter: Bakterielle Meningitis (BM); Biomarker; Diagnose; Krankheitsverlauf; Liquor cerebrospinalis; Outcome; Therapieansprechen.

Introduction

The diagnosis of bacterial meningitis (BM) relies on cerebrospinal fluid (CSF) examination performed after lumbar puncture (LP). Elevated white blood cells (WBCs) of $\geq$500 cells/mm^3, usually ranging between 1000 and 5000 cells/mm^3, a CSF-blood glucose ratio of $\leq$0.4, and a CSF lactate concentration of $\geq$3.5 mmol/L support the diagnosis [1, 2]. Furthermore, CSF protein is elevated in almost all cases of BM [2]. Because CSF cultures, being positive in 70% to 85% of patients without prior treatment, take up to 48 h for organism identification [2], treatment decisions are based on other diagnostic tools [3]. Out of several faster tests, the Gram stain examination is recommended for routine use in patients with suspected meningitis [2].

Occasionally, CSF findings are inconclusive and are unable to distinguish between BM and aseptic meningitis (AM), or CSF Gram stain and culture results are negative [2, 3]. Therefore, biochemical markers in CSF might contribute to diagnosis, especially owing to the importance of an early diagnosis and early appropriate antimicrobial treatment, which both have a crucial influence on survival of BM patients [4]. Furthermore, CSF biomarkers could support prediction of disease severity, treatment response, and clinical outcome.

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Materials and methods

Search strategy

A Medline search using the search terms 'bacterial meningitis marker' AND 'cerebrospinal fluid', limited to the time between 1 January 1990 and 1 October 2008 returned 199 references. Abstracts that primarily did not deal with BM and potential markers in the CSF of humans (e.g., carcinomatous meningitis, ventriculostomy-related infections, postoperative meningitis, experimental BM, treatment of BM) as well as those about tuberculous meningitis and neuroborreliosis were excluded, resulting in 61 abstracts. In addition, articles identified in reference lists of individual papers were selected if considered appropriate. Only original articles written in English or German were considered for this review.

Evidence was classified as classes I–IV and recommendations as levels A–C according to the scheme approved for the European Federation of Neurological Societies guidelines (EFNS). When only class IV evidence was available this could be recommended as a good practice point if it appeared appropriate [5]. In the case of insufficient description of the study design, evidence was considered as class III or lower.

Results

CSF biomarkers discriminating bacterial from aseptic meningitis

Occasionally, routine CSF findings are inconclusive and are unable to distinguish between BM and AM, or CSF Gram stain and culture results are negative [2, 3]. Therefore, an ideal biochemical marker should allow for the discrimination between these two types of meningitis in order to start causal treatment as early as possible.

Cytokines: tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-8 (IL-8) Cytokines play a role in the inflammatory reaction against the pathogen causing this bacterial infectious nervous disease [6, 7]. Accordingly, CSF levels of the cytokines TNF-α, IL-1β, IL-6, and IL-8 were significantly elevated in patients with BM compared to controls [8–20, 28]. They were also significantly higher compared to patients with AM [8, 10, 11, 13–19, 20–22] and showed sensitivities and specificities of 46.5%–93% and 81%–100% for discrimination of BM from AM, respectively [8–10, 14, 20, 22].

Acute phase proteins: C-reactive protein (CRP), α1-antitrypsin (AAT), α1-acid glycoprotein (AAG), haptoglobin (HPT), and ceruloplasmin (CER) CRP is secreted in response to a variety of inflammatory cytokines, mainly in response to IL-6, and is produced in many different types of inflammation [23]. It is the most widely used inflammatory marker to discriminate between bacterial and viral infections [24] and is predominantly measured in serum. Concerning meningitis, several studies revealed that CRP levels are significantly elevated in the CSF of BM patients compared to controls [25, 26], and compared to AM patients [25, 27]. With regard to diagnosis, CSF CRP showed sensitivities and specificities of 70%–100% and 90%–95%, respectively [26, 27]. Investigations of the acute phase proteins AAT, AAG, HPT, and CER also showed significantly higher values in BM than in AM patients with sensitivities and specificities of 50%–83% and 90%–97% for discrimination between these two meningitis types, respectively [27].

Cortisol Cortisol is an important anti-inflammatory mediator and cortisol concentrations were found to be significantly increased in BM patients compared to AM patients and controls. CSF cortisol levels showed a sensitivity of 82% and a specificity of 100% for discrimination of BM from AM [16].

Blood-brain barrier (BBB) associated proteins: soluble intercellular adhesion molecule-1 (sICAM-1) and matrix metalloproteinase 9 (MMP-9) Several proteins are involved in the immune reaction in BM, mediating leukocyte diapedesis through the BBB. Normally, leukocytes do not adhere to endothelial cells. However, when both are activated they express adhesion molecules such as ICAM-1 at their surfaces for rolling, adhesion, and transendothelial migration of leukocytes. Later, MMPs are needed for remodeling of the extracellular matrix (ECM) [28–31].

Investigations of sICAM-1, the soluble form of the transmembrane glycoprotein ICAM-1, revealed significantly increased values in the CSF of BM patients compared to AM patients [28, 32] and controls [28, 32–34]. One study even found elevated sICAM-1 CSF/serum ratios in BM, discriminating from AM and control individuals [32]. Whether elevation of sICAM-1 is a result of intrathecal production, reflected by an increased index, or as a result of severe BBB dysfunction, or owing to a combination of both is not clear [32, 35]. Furthermore, a study should be mentioned that found significant differences of sICAM-1 levels and sICAM-1 indices between BM and controls but not between BM and AM [33].

Concentrations of MMP-9 showed significantly increased values in BM patients compared to AM patients and controls [28].

Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) sTREM-1 is a cell surface molecule with augmented expression on phagocytes by exposure to bacteria or fungi. Consistently, CSF sTREM-1 levels were significantly higher in BM patients than in the AM and control groups. For discrimination between BM and AM, sensitivities of 73%–78% and specificities of 77%–100% were calculated [36, 37].

High mobility group box 1 (HMGB1) Concentrations of HMGB1, a proinflammatory mediator, were significantly higher in the CSF of BM patients than in the CSF of AM patients and control individuals [38].

Heat shock protein 72 (Hsp72) CSF levels of Hsp72 were significantly elevated in BM patients compared to AM patients and control subjects [38].

Macrophage colony-stimulating factor (M-CSF) M-CSF levels were significantly increased in the CSF of BM patients compared to AM patients and control individuals [39].

14-3-3 protein 14-3-3 protein, a marker for neuronal damage, was detected in the CSF of BM patients [40, 41]. Furthermore, one study reported a significant difference between the BM and the AM group [40].

Oxidative stress: nitrite, acrolein-lysine adducts (ALA), bilirubin derivates, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) The central nervous system (CNS) is vulnerable to effects of the oxidative stress owing to the brain high lipid content, which is the main target of the reactive oxygen species, and owing to its low antioxidant defense [42, 43]. Nitrite, a marker of nitric oxide production, ALA, bilirubin derivates, and 8-OHdG showed significantly higher levels in BM patients compared to AM patients [44–46] and control groups [44–47].

CSF biomarkers reflecting disease severity

TNF-α There are some lines of evidence showing that cytokines, which play a role in the inflammation cascade, lead to tissue destruction in BM [6]. Furthermore, the inflammatory reaction to the invading CNS pathogen rather than the pathogen itself appears to be largely responsible for brain damage [7]. Thus, there is evidence that TNF-α correlates significantly with disease severity [11, 21]. Levels of TNF-α correlated with bacterial density in CSF, consecutive febrile hospital days [21], and albumin quotient indicating the degree of BBB disruption [11]. In addition, initial high CSF TNF-α values were associated with seizures [21].

Cortisol CSF cortisol concentrations of BM patients correlated significantly with the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, the Sequential Organ Failure Assessment (SOFA) score, and the Glasgow coma scale (GCS) [16].

Platelet-activating factor (PAF) PAF, initially noted for its ability to promote platelet aggregation and thrombosis [48], also acts as a proinflammatory mediator that activates neutrophils [49, 50] and interacts with other cytokines such as TNF-α and IL-8 [51–53]. Because BM is an inflammatory disease, significantly higher concentrations of PAF were found in the CSF of BM patients compared to control individuals and correlated with bacterial density, CSF concentrations of lipopolysaccharide (LPS) and TNF-α, as well as with the Herson-Todd severity score [21].

Lipooligosaccharide (LOS) LOS concentrations in the CSF of patients with Haemophilus influenzae type b meningitis significantly correlated with bacterial density, glucose concentration, protein, IL-1β, neurological abnormalities during hospitalization, duration of secondary fever, and Herson-Todd score. Furthermore, LOS concentrations did not disappear in the CSF of patients who had seizures and longer duration of fever [54].

CSF biomarkers predicting clinical outcome

Cortisol CSF cortisol concentrations of BM patients correlated significantly with the Glasgow Outcome Scale (GOS) [16].

LOS Clinical outcome assessment revealed that patients with high LOS levels had significantly more long-term complications than those with low LOS concentrations, i.e., 28% of patients with high LOS levels had neurologic abnormalities compared to none of those with low LOS concentrations [54].

14-3-3 protein 14-3-3 protein levels correlated with clinical outcome, although comparability of studies is limited. One study found that patients who recovered cleared 14-3-3 protein from the CSF before discharge from the hospital, whereas patients who died never cleared the protein [41]. Another study described an association of 14-3-3 protein γ isoform levels on admission with clinical outcome [40].

Nitrotyrosine (NT) CSF concentrations of NT, a marker for the formation of reactive nitrogen species, showed a significant elevation in BM patients; and high CSF NT levels were associated with an unfavorable outcome, determined by the GOS [55].

CSF biomarkers indicating treatment response

Cytokines: TNF-α and IL-1β TNF-α and IL-1β play a key role in the inflammatory reaction in BM. Several studies found that the concentrations of TNF-α and IL-1β are increased in this infectious nervous disease but decrease with response to treatment and, thus, with clinical improvement [11, 13, 14, 21].

β-Amyloid 42 (Aβ42) BM patients were found to have significantly decreased values of Aβ42 in their CSF compared to controls. After successful antibiotic treatment, there was an increase and finally a normalization of CSF Aβ42 levels [56].

Oxidative stress: nitrite and ALA Nitrite and ALA concentrations, which were elevated in the CSF of BM patients, decreased significantly as the patients started to respond to antibiotic treatment [44, 45].

CSF biomarkers lacking evidence of clinical relevance

Levels of lipopolysaccharide binding protein (LBP) [8, 57], α2-macroglobulin (α2M) [58], ferritin [59, 60], and serum amyloid A protein (SAA) [25] were found to be elevated in the CSF of BM patients compared to AM patients. Fibronectin correlated with treatment response [61], and lipoteichoic acids and teichoic acids (LTA/TA) with treatment response and clinical outcome [62]. However, evidence was insufficient to classify these biomarkers according to the EFNS guidelines.

The results of studies on neopterin [63, 64], biopterin [63], matrix metalloproteinase 2 (MMP-2) [28], procalcitonin (PCT) [25, 65–67], τ [56, 68], and cyclic guanosine monophosphate (cGMP) [69] could not provide evidence that these biomarkers are clinically useful.

CSF concentrations of SL-selection [34], soluble endothelial leukocyte adhesion molecule-1 (sELAM-1) [34], muramic acid (MA) [70], N-acetylmuramyl-L-alanine amidase (NAMLAA) [71], lysozyme (LZM) [71, 72], human guanylate binding protein 1 (hGBP-1) [73], soluble TNF-related apoptosis-inducing ligand (sTRAIL) [74], Fas (APO-1/CD95) [75], β_2-microglobulin (β2M) [59], natural peroxynitrite scavenger uric acid, allantoin, and ascorbic acid [55] were significantly increased in BM patients compared to controls, but these studies did not present results regarding discrimination from AM or prediction of disease severity, clinical outcome, and treatment response (not shown in Table 1). Thus, evidence of these markers only suggests their role in the pathomechanism of BM without clinical relevance.

The classification of biomarkers according to EFNS guidelines is summarized in Table 2. An overview of evidence of CSF biomarkers in BM regarding diagnosis and prediction of disease severity, clinical outcome, and treatment response is shown in Table 1.

Conclusions

BM is a serious infectious disease of the CNS. The inflammatory reaction to the invading pathogen, rather than the pathogen itself, appears to be largely responsible for brain damage [7] and leads to the release of biomarkers into the CSF. Elevated levels of several inflammatory mediators, such as TNF-α, IL-1β, IL-6, IL-8, sTREM-1, and HMGB1, in the CSF of BM patients compared to AM patients and controls were consistently found. Acute phase proteins, cortisol, the BBB associated proteins sICAM-1 and MMP-9, Hsp72, M-CSF, markers for oxidative stress, such as nitrite, ALA, bilirubin derivates, and 8-OHdG, and 14-3-3 protein, a marker for neuronal damage, also showed significantly elevated levels. Furthermore, there are several lines of evidence that disease severity correlates with levels of TNF-α, cortisol, PAF, and LOS; clinical outcome with levels of cortisol, LOS, 14-3-3 protein and nitrotyrosine; and response to antibiotic treatment with levels of TNF-α, IL-1β, Aβ42, nitrite, and ALA, respectively.

The diagnosis of BM relies on clinical signs and symptoms as well as on CSF examination. Occasionally, the discrimination between BM and AM is difficult owing to inconclusive CSF findings and negative CSF Gram stain and culture results [2, 3]. CSF WBC counts have wide variations. Considerably lower cell counts are observed in children [76] and in immunocompromised patients [77], and even acellular CSF has been reported in immunocompetent patients with meningococcal [78] and pneumococcal meningitis [79]. High numbers of neutrophils, which is normal in the CSF of patients with BM, can also be observed in the early course of AM [80]. Furthermore, the elevation of total protein content is nonspecific for BM and occurs in many other inflammatory neurological disorders. The elevation of CSF lactate concentration can also be caused by several other factors, such as metabolism of CSF leukocytes [2]. Regarding diagnostic microbiology, Gram staining shows a broad variation of sensitivity (50%–90%) [81], and CSF cultures, which are the gold standard for diagnosis of BM and positive in 70%–85% of patients without prior treatment, take up to 48 h for organism identification and, thus, they are not applicable for early treatment decisions [2]. Furthermore, sensitivity of CSF cultures is reduced when antibiotics have been administered before LP [82]. In cases of suspected meningitis and inconclusive findings, new CSF biomarkers could contribute to diagnosis and support discrimination between BM and AM. Based on the above reports, it seems that BM causes a more severe inflammation than AM and leads to the release of different amounts of several biomarkers. Best evidence exists for several cytokines, particularly TNF-α, acute phase proteins, mainly CRP, and nitrite. Regarding clinical relevance, prospective studies are required to investigate the role of these biomarkers in BM cases, which present inconclusive CSF findings, such as low WBC count, acellular CSF, and negative microbiological results.

Disease severity of BM patients was assessed by several different methods, e.g., APACHE II score, SOFA score, GCS, and Herson-Todd severity score or by clinical features such as bacterial density in the CSF, consecutive febrile days, duration of hospitalization, or occurrence of seizures. Clinical outcome was consistently assessed either by the GOS or the classification of patients in survivors and non-survivors. Treatment response was described by clinical improvement. Best evidence exists for TNF-α for estimation of disease severity and treatment response as well as for 14-3-3 protein for clinical outcome prediction. However, in addition to common clinical assessments, CSF biomarkers

Table 1 Evidence of cerebrospinal fluid biomarkers in bacterial meningitis regarding discrimination from aseptic meningitis and prediction of disease severity, clinical outcome, and treatment response.

Marker	No. of patients					Specification of controls	Assay	Significant difference between BM and			Diagnostic		Predictor of			Evidence class
	Ref. No	BM	AM	MM	Co.			AM	MM	Co.	Sensitivity, %	Specificity, %	Severity	Outcome	Treatment response	
TNF-α		*318*	*347*		*382*						*74–84.2*	*81–100*				
	8	40	46		22	NID	CIA	y		y	83.7	91.4				III
	21	38	8		27	OND, FNM, ENC	ELISA	y					+	–	+	II
	22	51	78		20	Healthy	IRMA	y		y	82	94				III
	10	20	22		42	Healthy, OND	ELISA	y		y	84.2	100				III
	11	48	20		66	Healthy, NIND	ELISA	y		y			+		+	II
	12				24	NID				y						III
	13	11	20		11			y		y					+	III
	14	23	26		95	FNM	IA	y		y	74	81			+	III
	15	11	50		15	FNM	RIA	y		y						III
	16	47	37		13	Healthy	CBAK	y		y						II
	17	29	40		47	Healthy		y		y						II
IL-1β		*142*	*144*		*240*						*78–91.2*	*93.6–99*				
	8	40	46		22	NID	CIA	y		y	91.2	93.6				III
	10	20	22		42	Healthy, OND	ELISA	y		y	78.9	99				III
	11	48			66	Healthy, NIND	EIA	y		y			–		+	II
	14	23	26		95	FNM	EIA	y		y	78	96			+	III
	15	11	50		15	FNM	RIA	y		y						III
IL-6		*113*	*118*		*66*						*60–93*	*93.1–94*				
	8	40	46		22	NID	CIA	y		y	93	93.1				III
	13	11	20		11			y		y					+	III
	16	47	37		13	Healthy	CBAK	y		y						II
	9	15	15		20	NIND	ELISA			y	γ60	γ94				III
	18						RIA	y		y						III
IL-8		*141*	*166*		*87*						*46.5–81*	*91.4–92*				
	8	40	46		22	NID	CIA	y		y	46.5	91.4				III
	19	7	11		11	Gastroenteritis	ELISA	y		y						III
	13	11	20		11			y		y						III
	28	5	39		22	NIND	ELISA	n		y						II
	16	47	37		13	Healthy	CBAK	y		y						II
	20	31	13		8	NID	ELISA	y		y			+			III
IFN-γ																
	15	11	50		15	FNM	RIA	y		n						III
sICAM-1		*90*	*75*		*144*											
	28	5	39		22	NIND	ELISA	y		y						II
	35	31			33	Healthy	ELISA			y						III

Table 1 (Continued)

Marker	No. of patients					Specification of controls	Assay	Significant difference between BM and			Diagnostic		Predictor of			Evidence class
	Ref. No	BM	AM	MM	Co.			AM	MM	Co.	Sensitivity, %	Specificity, %	Severity	Outcome	Treatment response	
	32	24	13		50	NID	ELISA	y		y				+		III
	33	9	23		18	NIND	EIA	n		y						III
	34	21			21	Healthy	IA			y						III
MMP-2																
	28	5	39		22	NIND	ELISA	n		n						II
MMP-9																
	28	5	39		22	NIND	ELISA	y		y						II
CRP		*67*	*50*	*3*	*42*						*70–100*	90–95				
	25	7	20	3	12	OND	IA	y		y						III
	26	30			30	Non-meningitis	LA			y	100	95				II
	27	30	30				Nephelometry	y			70	90				II
AAT																
	27	30	30				Nephelometry	y			83	90				II
AAG																
	27	30	30				Nephelometry	y			67	90				II
HPT																
	27	30	30				Nephelometry	y			50	97				II
CER																
	27	30	30				Nephelometry	y			80	90				II
PCT		*56*	*114*	*3*	*37*						*55*	*100*				
	25	7	20	3	12	OND	LUMI	n	n	n						III
	65	6	12					n		n						II
	66	20	25				LUMI	y			*55*	*100*				II
	67	23	57		25	Non-meningitis	LUMI	n		n						II
Neopterin		*19*	*52*		*89*											
	63	7	34		72	Healthy		y						–		III
	64	12	18		17	Febrile convulsion	RIA	y								III
Biopterin																
	63	7	34		72	Healthy		y						–		III
PAF																
	21	38	8		27	OND, FNM, ENC	See ref.			y			+			II
Cortisol																
	16	47	37		13	Healthy	RIA	y		y	82	100	+	+		II

Table 1 (Continued)

Marker	No. of patients					Specification of controls	Assay	Significant difference between BM and			Diagnostic		Predictor of			Evidence class
	Ref. No	BM	AM	MM	Co.			AM	MM	Co.	Sensitivity, %	Specificity, %	Severity	Outcome	Treatment response	
LBP		*60*	*46*		*41*						*81.4*	*87.7*				
	8	40	46		22	NID	CIA	y			81.4	87.7				III
	57	20			19	OND, JIA, VI	CIA			n						II
LTA/TA																
	62	30					EIA							+	+	III
LOS																
	54	38					CLA						+	+		II
M-CSF																
	39	4	10		14	Non-meningitis	ELISA	y		y						II
sTREM-1		*101*	*14*		*15*						*73–78*	*77–100*				
	36	92	8		9	Healthy	ELISA	y		y	73	77			-	III
	37	9	6		6		ELISA	y		y	78	100				II
HMGB1																
	38	13	38		46	Non-meningitis	Western blot	y		y						II
Hsp72																
	38	13	38		46	Non-meningitis	Western blot	y		y						II
Ferritin		*52*	*102*		*519*						*100*	*78*				
	59	5	39		78	Non-meningitis	LPIA	y		y	100	78	+		+	III
	60	47	63		441			y		y						III
cGMP																
	69	9	15		29	NIND	EIA	n		y						III
Fibronectin																
	61	11	9		25	Healthy	Turbidimetry	y		y					+	III
α2M																
	58	10	25	5	13	NIND	ELISA	y	y	y						III
SAA																
	25	7	20	3	12	OND	EIA	y		y						III
14-3-3		41	12													
	40	29	12				See ref.	y						+		II
	41	12					See ref.							+		II
Aβ42																
	56	8	10		18		ELISA			y					+	II

Table 1 (Continued)

Marker	No. of patients					Specification of controls	Assay	Significant difference between BM and			Diagnostic		Predictor of			Evidence class
	Ref. No	BM	AM	MM	Co.			AM	MM	Co.	Sensitivity, %	Specificity, %	Severity	Outcome	Treatment response	
τ		15	17		65											
	68	7	7		47	OND	ELISA	n		n						III
	56	8	10		18		ELISA	n		n						II
Nitrite		*65*	*40*		*99*											
	45	10	18		14	FNM	Diazotization	y		y					+	II
	44	11	7		26	Non-meningitis	Diazotization	y		y					+	II
	47	35			30	ALL, NHL in rem.	CM			y						III
	69	9	15		29	NIND	Griess reaction	n		n						III
Nitrate		*44*	*15*		*59*											
	47	35			30	ALL, NHL in rem.	See ref.			y						III
	69	9	15		29	NIND	Griess reaction	n		n						III
NT																
	55	15			14	OND	ELISA			y				+		II
ALA																
	45	10	18		14	FNM	ELISA	y		y					+	II
BiliDer																
	45	10	18		14	FNM	ELISA	y		y						II
8-OHdG																
	46	16	31		16	FNM	ELISA	y		y						II

y, yes; n, no; +, predictor; –, no predictor, empty cells denote that these parameters were not investigated; data in italic font summarize the number of patients per group and present the range of sensitivity and specificity of the marker below. ALL, acute lymphatic leukemia; CBAK, cytometric bead array kit; CIA, chemiluminescence immunoassay; CLA, chromogenic limulus assay; CM, colorimetric method; Co., control group; EIA, enzyme immunoassay; ELISA, enzyme linked immunosorbent assay; ENC, encephalitis; FNM, fever-non-meningitis; IA, immunoassay; IRMA, immunoradiometric assay; JIA, juvenile idiopathic arthritis; LA, latex agglutination; LPIA, latex photometric immunoassay; LUMI, immunoluminometric assay; MM, mycotic meningitis; NHL, non-Hodgkin's lymphoma; NID, non-inflammatory disease; NIND, non-inflammatory/infectious neurological disease; OND, other neurological disease; rem., remission; RIA, radioimmunoassay; VI, viral infection.

Table 2 Biomarker classification according to the European Federation of Neurological Societies guidelines.

	Significant difference between BM and AM	Predictor of		
		Disease severity	Outcome	Treatment response
Cytokines				
TNF-α	A	A		B
IL-1β	B			B
IL-6	B			
IL-8	B			
Cortisol	B	B	B	
Acute phase proteins				
CRP	B			
AAT	B			
AAG	B			
HPT	B			
CER	B			
BBB associated proteins				
sICAM-1	C			
MMP-9	C			
sTREM-1	C			
HMGB1	C			
Hsp72	C			
PAF		B		
M-CSF	C			
LOS		B	B	
14-3-3 protein	C		B	
Aβ42				C
Oxidative stress				
Nitrite	B			C
Acrolein-lysine adducts	C			C
Bilirubin derivates	C			
8-OHdG	C			
Nitrotyrosine			C	

Level A, established as useful/predictive marker; level B, established as probably useful/predictive marker; level C, established as possibly useful/predictive marker.

hardly provide additional information for physicians, specifically in individual clinical decision-making, whereas they could possibly serve as examiner-independent tools in study settings.

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