

# EFNS guidelines on disease-specific CSF investigations

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We reviewed the literature for disease-specific markers in cerebrospinal fluid (CSF) and evaluated their diagnostic and prognostic relevance in neurological diseases. High tau protein in combination with low amyloid  $\beta$  levels has a high sensitivity (80%) and specificity (90%) for Alzheimer's disease (AD) against normal aging and can predict conversion of mild cognitive impairment to AD. The detection of 14-3-3 has a high sensitivity (80–90%) and specificity (90%) for the diagnosis of CJD. Low or undetectable CSF hypocretin-1 (orexin-1) levels constitute a diagnostic biomarker for narcolepsy with cataplexy. Detection of beta-2-transferrin indicates CSF contamination in oto- and rhinorrhoe with a sensitivity of >79% at a specificity of 95% similar to the beta-trace protein (sensitivity >90%, specificity 100%). However, beta-trace protein is faster and cheaper to perform. Possible future biomarkers are: elevated levels of vascular endothelial growth factor are relatively sensitive (51–100%) and specific (73–100%) for leptomeningeal metastases from solid tumors and are associated with a poor prognosis in this condition. Elevated CSF neurofilament (Nf) levels probably reflect acute neuronal degeneration. The prognostic value of CSF Nf levels is highest in acute conditions such as subarachnoid hemorrhage, acute optic neuritis and neuromyelitis optica.

## Introduction

Investigation of the cerebrospinal fluid (CSF) in neurological diseases has a long history and a small number of molecules have become the standard repertoire in routine CSF work-up such as total protein, glucose, cell count and differentiation, as well as quantitative and qualitative detection of immunoglobulins [1]. In recent years, the search for biomarkers – a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention, as defined by the Biomarkers Definition Working Group [2] – in body fluids including the CSF has increased substantially. In addition to the guidelines on routine CSF investigations [1] we wanted to evaluate

newer CSF markers with respect to their disease specificity or prognostic relevance.

## Search strategy

### Tau protein, amyloid beta, 14-3-3 and myelin basic protein

PubMed was searched up to January 2008 for key words including 'CSF', 'tau', 'amyloid', '14-3-3', 'myelin basic protein', and 'mbp', which yielded 1785 hits. Only papers in English and relating to adult clinical neurology were considered for further analysis.

### Hypocretin

A Medline search using the search terms cerebrospinal fluid (CSF), narcolepsy, sleep disorder, hypocretin and orexin was conducted. The search was limited to the time between 1 January 1980 and 1 May 2008. The key words were cross-referenced as follows: ('cerebrospinal fluid' or 'CSF') AND ('hypocretin or orexin') AND

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(‘narcolepsy OR sleep disorder OR cataplexy’). The search returned 73 documents. Abstracts which primarily did not deal with human sleep disorders, case reports, reviews, comments and original articles, which were not published in English, were excluded. The remaining 13 articles were further analyzed.

### Beta 2 transferrin

(Cerebrospinal fluid OR CSF) and (beta 2 transferrin) and (otorrhea OR otorrhoea OR rhinorrhoea OR rhinorrhea) NOT beta trace resulted in 60 abstracts. Reviews and reports not dealing with diagnostic issues or with other fluids than CSF were excluded resulting in 42 articles.

### Beta trace protein (prostaglandin D synthase)

(Beta-trace protein OR Prostaglandin D Synthase) and (otorrhea OR otorrhoea OR rhinorrhoea OR rhinorrhea) NOT beta 2 transferrin resulted in 16 abstracts.

### Vascular endothelial growth factor

PubMed Search for (‘VEGF’ OR ‘vascular endothelial growth factor’) AND ‘CSF’; limits: 1980 until May 2008, Language German or English, reports on humans resulted in 51 Abstracts. Of those four reviews, 21 papers not related to diagnostic VEGF testing in CSF, and four pediatric reports were excluded. The remaining 22 papers were included for evaluation.

### Neurofilaments

PubMed was searched up to January 2008 for key words including CSF, neurofilament (Nf), patients, which yielded 91 hits. Articles dealing with adult patients were included (children excluded). Articles excluded were: articles focussing on anti-Nf antibodies, serum analysis only, animal studies, reviews, case reports or method description rather than biomarker investigation in patients, animal studies and articles that were not in English.

Evidence was classified as class I–IV and recommendations as level A–C according to the scheme agreed for EFNS guidelines [3] with class I and level A being the strongest classes of evidence/recommendation.

In a conference held in March 2006, various potential CSF biomarkers and methods of detection were discussed by the members of the taskforce including CSF proteomics, markers of axonal damage focusing on different subsets of Nfs, neurotransmitters, autoantibodies, anti-neural antibodies, ferritin, Amyloid- $\beta$  (A $\beta$ ),

tau and hyperphosphorylated-tau, 14-3-3 protein, myelin basic protein, sulfatid, beta-2-transferrin, VEGF, and S-100. Of those, only the markers discussed below were felt to be relevant enough to receive further attention for the present report.

The Cochrane database and guideline papers of the American Academy of Neurology (AAN) were searched for all above topics. No relevant publication was found.

## CSF markers recommended for use in clinical routine

### Tau protein and amyloid- $\beta$ peptide

Tau proteins are microtubule-associated proteins important for the maintenance of axonal microtubules. Hyperphosphorylated tau (P-tau) is present in neuritic tangles in Alzheimer’s disease (AD). Amyloid- $\beta$  (A $\beta$ ) peptides are derived from cleavage of the amyloid precursor protein by  $\beta$ - and  $\gamma$ -secretases, resulting in the A $\beta_{1-40}$  and A $\beta_{1-42}$  peptide fragments. A $\beta_{1-42}$  has a strong tendency to aggregate, and is a major component of amyloid plaques in AD. Increased CSF concentrations of tau and P-tau and low CSF concentrations of A $\beta_{1-42}$  are typical findings in AD, have been reviewed by a task force of the World Federation of Societies of Biological Psychiatry, and have been incorporated in the latest research criteria for the diagnosis of AD [4–7]. Tau proteins and A $\beta$  peptides can be easily measured by commercially available sandwich ELISA, but A $\beta$  measurements should be interpreted with some caution (see below).

At a specificity of 90%, the sensitivity for AD according to clinical criteria of the most commonly used tau immunoassay is approximately 80%, and the sensitivity of A $\beta_{1-42}$  is 80–90% (class I) [4]. The sensitivity for the diagnosis of AD of P-tau assays, based on immunoassays recognizing tau phosphorylated on different serine and threonine residues, is more variable (class II) [4,8,9]. The combination of tau and A $\beta_{1-42}$  has been assessed in several studies, and has been found to give a high sensitivity and specificity against normal aging and psychiatric disorders, whereas the specificity against other diseases is lower (class II) [10,11]. Increases in tau and low A $\beta_{1-42}$  concentrations are also found in stroke, trauma and other diseases with a neurodegenerative component (Table 1). However, at least for stroke, P-tau levels have been reported as being normal [12,13]. P-tau may provide better specificity against dementia with Lewy bodies and frontotemporal dementia, but the phosphorylation sites providing the best discrimination may differ from one disease to another [14–18]. Low concentrations of A $\beta_{1-42}$  and

**Table 1** Summary of studies of total tau and amyloid  $\beta_{1-42}$  ( $A\beta_{1-42}$ ) peptide in cerebrospinal fluid in diseases other than Alzheimer dementia

Disease and results	Evidence classification	Reference
Multiple sclerosis (MS)		
Increase in tau and P-tau in MS	Class II	[62,169–172]
Normal tau in most MS patients	Class II	[173]
Dementia with Lewy bodies (LBD)		
Lower $A\beta_{1-42}$ in LBD	Class II	[10,174]
Increase in tau in LBD	Class III	[175,176]
Normal tau and $A\beta_{1-42}$ in LBD	Class II	[177]
Lower $A\beta_{1-42}$ and increased tau in LBD	Class II	[178,179]
Semantic dementia (SD)		
Increase in tau in SD	Class III	[180]
Frontotemporal dementia (FD)		
Increase in tau in FD	Class III	[175,176]
Increase in tau in FD	Class II	[181]
Increase in tau, decrease in $A\beta_{1-42}$	Class II	[182]
Low $A\beta_{1-42}$ in FD	Class III	[183]
Parkinson disease (PD)		
Lower $A\beta_{1-42}$ to $A\beta_{1-37}$ ratio in PD with dementia	Class III	[29]
Normal $A\beta_{1-42}$ in PD	Class II	[184]
Lower $A\beta_{1-42}$ and higher tau in PD with dementia, intermediate values in PD	Class II	[185]
Low $A\beta_{1-42}$ in PD	Class III	[183]
Multiple system atrophy (MSA)		
Higher tau in MSA than in PD	Class II	[186]
Higher tau in MSA than in idiopathic cerebellar ataxia	Class II	[187]
Low $A\beta_{1-42}$ in MSA	Class II	[184]
Corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP)		
Higher tau in CBD than in PSP	Class II	[176,188]
Normal $A\beta_{1-42}$ in PSP	Class II	[184]
Low $A\beta_{1-42}$ in CBD and PSP	Class III	[189]
Vascular dementia (VD) and stroke		
Increase in tau in VD	Class II	[10]
Increase in tau in stroke	Class II	[12]
Increase in tau, low $A\beta_{1-42}$ and normal P181-tau in VD	Class II	[13,190]
Increase in tau, normal $A\beta_{1-42}$ and normal P181-tau in VD	Class II	[190]
Neuromuscular diseases		
Increased tau in amyotrophic lateral sclerosis	Class II	[137]
Low $A\beta_{1-42}$ in ALS	Class III	[183]
Increased tau in Guillain-Barré syndrome, correlation with prognosis	Class II	[191]
AIDS-dementia complex (ADC)		
Low $A\beta_{1-42}$ and high tau in ADC	Class II	[192]
Head injury		
Increased tau in head injury	Class III	[193,194]
Decreased $A\beta_{1-42}$ in head injury	Class III	[193,195]
Normal pressure hydrocephalus (NPH)		
Low tau, P-tau and $A\beta_{1-42}$ in NPH	Class II	[196]
Increased tau in NPH	Class III	[176,197]

dramatic increases in tau without a similarly strong increase in P-tau, is observed in CJD (class I) [19–27]. In contrast, tau and P-tau increase in parallel in variant CJD (class III) [28]. The discrimination between different tau and  $A\beta$  isoforms in CSF may be useful for the discrimination between different disease processes, but larger studies are needed to address this in detail [29–33].

A special indication for CSF studies is the identification of patients with mild cognitive impairment with CSF findings typical of AD [4], as there is class I evidence that such patients may be at increased risk of progression to AD [34–43]. When interpreting the results of  $A\beta_{1-42}$  measurements, it should be taken into account that concentrations may decrease following glucocorticoid treatment (class III) [44] and show pronounced diurnal fluctuations [45], and erroneously low values of  $A\beta_{1-42}$  are found if the CSF is not sampled and handled optimally [46–48]. Indeed, freezing and prolonged storage impairs the discriminatory value of ELISA measurements of  $A\beta$  [49]. Furthermore, age-dependent reference values should be used for tau measurements, whereas  $A\beta_{1-42}$  values are independent of age. In one study, the reference value for  $A\beta_{1-42}$  was above 500 ng/l; for tau the reference value was <300 ng/l in patients from 21 to 50 years of age, <450 ng/l from 51 to 70 years of age, and <500 ng/l from 71 to 93 years of age [50].

#### Recommendation

In studies assessing the sensitivity and specificity of CSF markers for AD, the diagnosis has not been confirmed neuropathologically.  $A\beta_{1-42}$  values should be interpreted with some caution. There is, however, evidence that tau, p-tau and  $A\beta_{1-42}$  measurements have high sensitivity and specificity for discriminating AD against normal aging, and may identify patients with mild cognitive impairment at an increased risk of progression to AD (level A rating). The latter finding is of clear importance if therapies affecting the disease course of AD become available in the future.

#### 14-3-3 protein

14-3-3 is a ubiquitous protein with the highest concentrations found in brain [51]. Mostly, it is determined qualitatively by western blot techniques, although a quantitative ELISA method is available. An initial study showed the presence of 14-3-3 in CSF from 96% of 71 patients with Creutzfeldt-Jakob disease (CJD) (class II) [52]. In a prospective study including 805 patients with neuropathologically confirmed CJD the sensitivity of a positive 14-3-3 test was 94%, the specificity was 84%, and the 14-3-3 test was superior to EEG studies (class I) [53].

High sensitivity and specificity of 14-3-3 has also been found in other studies (class I) [54–58]. Increases in 14-3-3 protein are less frequent in new variant CJD (class III) [59], in familial forms of spongiform encephalopathies and in infrequent subtypes of CJD (class III) [56,60,61]. Furthermore, increases in 14-3-3 protein are less frequent early and late in the course of sporadic CJD and early in the course of iatrogenic (growth hormone-induced) CJD (class III) [14,43,62]. Positive results of the 14-3-3 test have also been reported in other dementias, cerebrovascular disease, metabolic and hypoxic encephalopathies, brain metastases and CNS infections (class III) [27,27,52,55,58,63,64]. The detection of 14-3-3 in CSF has been reported to indicate a poor prognosis in patients with transverse myelitis, clinically isolated syndromes suggestive of MS and MS (class II) [65–67]; another study did not confirm these findings (class III) [68].

#### Recommendation

The detection of 14-3-3 has been reported to have a high sensitivity (94%) and specificity (84%) for the diagnosis of CJD in patients with clinical findings suggestive of this disease, but 14-3-3 can also be detected in CSF from patients with more common diseases (level A rating).

#### Hypocretin-1 (orexin-1) levels in narcolepsy

Human narcolepsy is a sleep disorder which affects up to 0.5% of the population. It is characterized by hypersomnia, cataplexia, sleep paralysis and hypnagogic hallucinations [69]. The full spectrum of symptoms occurs only in a minority of patients. The disease is strongly associated with the HLA allele DQB1\*0602. Based on animal models it is believed that narcolepsy is caused by a dysfunction of the hypocretin-1 (orexin-1) neurotransmitter system in the posterior and lateral hypothalamus. In human narcolepsy CSF hypocretin-1

(orexin-1) levels were reported to be low or absent. Thirteen studies were used to evaluate the role of CSF hypocretin-1 (orexin-1) in sleep disorders. Three studies were excluded from the analysis because of low patient numbers or lack of control groups [70–72]. The study Mignot *et al.* included patients from two previous studies, which were therefore also excluded from the analysis [73,74]. In all studies hypocretin-1 (orexin-1) levels in CSF were measured by radio immunoassay (RIA). Cut-off points ranged from 202 to 110 pg/ml. In most patients with narcolepsy with cataplexy orexin/hypocretin concentrations were low (sensitivities between 66% and 100%), in the majority of patients even below the detection limit of 40 pg/ml of the RIA (Table 2). This was in particular true for patients with HLA DQB1\*0602 status [72]. Low levels of hypocretin-1 (orexin-1) were also found in a minority of narcolepsy without cataplexy patients and other neurological disease (specificities between 92% and 100%) [75].

#### Recommendation

Based on three class II and several class III and IV studies, hypocretin-1 (orexin-1) levels of < 110 pg/ml in CSF can be classified as a diagnostic biomarker for narcolepsy with cataplexy (level A recommendation, in compliance with the International Classification of Sleep Disorders). The value of measuring hypocretin-1 (orexin-1) in CSF of other sleep disorders is controversial.

#### Beta-2-transferrin

Transferrin is a polypeptide of the  $\beta$ -globulin family, which is involved in ferrous ion transport. Beta-2-transferrin (synonyms: asialotransferrin, tau-fraction) is a transferrin form, without sialic acid side chains. In healthy human beings beta-2-transferrin has only been demonstrated in CSF, perilymph and aqueous humor of the eye, but not in serum [76,77]. In patients with

**Table 2** Summary of the results from hypocretin-1/orexin-1 levels in narcolepsy

Sensitivity		Prevalence in controls <sup>a</sup>		Cut-off (pg/ml)	Class of evidence	Reference
With cataplexy	Without cataplexy	ND including SD <sup>b</sup>	ND without SD <sup>c</sup>			
69.3% <sup>d</sup> (137)	14.3% (21)	1.7% (228)	0% (47) <sup>e</sup>	110	III <sup>f</sup>	[198]
91.7% (48)	40% (15)	0% (10)	0% (50) <sup>e</sup>	110	II	[199]
81.8 (9)	0% (5)	8.3% (12)	nd	194	III	[200]
70.6% (17)	0% (9)	nd	0% (15) <sup>h</sup>	200	IV	[201]
100% (14)	100% (10)	0% (24) <sup>e</sup>	nd	100	IV	[202]
88.5% (26)	11.1% (9)	0% (103)	nd	110	II	[203]
66.0% (47)	0% (7)	0% <sup>i</sup> (10)	nd	209 pg/dl	II	[204]

All hypocretin-1/orexin-1 measurements were performed by RIA; numbers in parenthesis refer to total number of investigated subjects; <sup>a</sup>100% minus these figures result in specificities; <sup>b</sup>Neurological disease including other sleep disorders; <sup>c</sup>Neurological disease without sleep disorders; <sup>d</sup>including patients with atypical cataplexia; <sup>e</sup>patients who had no sleep disorder; <sup>f</sup>samples were included before in other studies <sup>g</sup>neurological diseases and pregnant women; <sup>h</sup>patients who underwent back surgery; <sup>i</sup>idiopathic hypersomnia.

chronic liver diseases [78,79], inborn error of glycoprotein metabolism [80] and persons with a genetic variant of transferrin [78,81,82] beta-2-transferrin can be also found in the blood.

Due to shortcomings of earlier methods (high resolution electrophoresis) [83,84] an isoelectric pH-focusing method was developed followed by immunofixation and silver staining, for which as little as 5 µg total protein is necessary (class III). Various sources of error in use of beta-2-transferrin analysis for diagnosing cerebral spinal fluid leaks were described [85], such as transferrin isoforms especially in saliva with electrophoretic mobility similar to that of beta-2-transferrin (class IV) [86].

In combination with other methods like high resolution computed tomography the detection of beta-2-transferrin in CSF contaminated nasal discharges and blood was able to confirm oto- and rhinorrhoe of different etiologies [83,84,87–94] (all class III, except one class IV) and used to monitor patients after surgical reconstruction of the frontal skull base (class III) [95]. The test sensitivity varied between 79% and 100% (class III) [94,96,97] at a specificity of about 95% (class III) [97]. Venous blood sampled at the same time as CSF may exclude false positive results due to inborn errors of glycoprotein metabolism, genetic variants of transferrin or chronic liver disease (class IV) [81,92].

#### Recommendation

Measurement of beta-2-transferrin in combination with computed tomography of the skull detects CSF contamination in oto- and rhinorrhoea with high sensitivity (79–100%) at a specificity of 95% (level B recommendation).

#### Beta-trace protein, β-TP (prostaglandin D synthase, PGDS)

β-TP (molecular mass, 25 kDa) is one of the most abundant locally synthesized proteins in the CSF [98,99]. Based on amino acid sequencing, it has been identified as prostaglandin D synthase (PGDS) which is a member of the lipocalin superfamily composed of

various secretory lipophilic ligand-carrier proteins [100]. Within the central nervous system, β-TP has been localized by immunohistochemistry and *in situ* hybridization mainly in choroid plexus and leptomeninges [101]. Concentrations of β-TP in CSF ranged between 8 and 40 mg/l (age-dependent) and between 0.4 and 1.5 mg/l in serum. Because of its relatively high CSF concentration β-TP has been explored for the diagnosis of oto- and rhinoliquorrhea [102–104].

For quantitative nephelometric analysis, a sample volume of at least 5 µl is needed which is diluted with a dilution buffer to a total volume of 500 µl. The detection range of the assay is between 0.25 and 15.8 mg/l, the detection limit is at 2.5 mg/l. The test requires <15 min having the advantage of intraoperative use and repeated frequent testing, e.g. evaluation of treatment success of CSF fistula repair [105].

Highest sensitivities and specificities as well as accuracy values were reported using a cut-off value of 1.31–6 mg/l [103,106,107] (Table 3, twice class II, one class III study). In addition, a secretion/serum ratio of β-TP has been introduced with a normal range below 1.57 yielding 100% specificity [108]. A cut-point at 1.11 mg/l for beta-trace protein gave the best trade-off between high sensitivity and high specificity when including the secretion/serum ratio [102]. In direct comparison to beta-2-transferrin the sensitivity and specificity values to detect CSF contamination in oto- and rhinorrhoea were similar (class II) [102,103].

A limitation of the β-TP assay is that in patients with renal insufficiency and bacterial meningitis levels may substantially increase in serum and decrease in CSF, respectively. Hence, its use in such instances should be associated with cautious interpretation of the results.

#### Recommendation

The β-TP test allows a quantitative detection of CSF fistulas with a high sensitivity and specificity (level A recommendation) in combination with computed tomography and clinical investigations. Due to procedural advantages β-TP should be used as first line

**Table 3** Different cut-off values have been evaluated for diagnostic accuracy of CSF fistula (beta-trace protein)

Sensitivity	Specificity	Rhino-/otorrhoea cut-off <sup>a</sup>	Normal nasal secretion, mean, (range) <sup>a</sup>	Serum mean, (range) <sup>a</sup>	CSF mean, (range) <sup>a</sup>	Class of evidence	Reference
92%	100%	> 6 <i>n</i> = 33	0.9 <i>n</i> = 107	0.5 <i>n</i> = 34	11.1 <i>n</i> = 20	II	[106]
Not reported	Not reported	> 0.35 <i>n</i> = 20	0.016 (0.0–0.12) <i>n</i> = 29	0.6 (0.38–0.86) <i>n</i> = 132	18.4 (9.4–29.2) <i>n</i> = 132	III	[107]
93%	100%	> 1.31 ( <i>n</i> = 53)	0.4 (0.22–1.69) <i>n</i> = 160	0.6 (0.12–1.44) <i>n</i> = 116	19.6 (11.5–32.6) <i>n</i> = 19	II	[103]

CSF, cerebrospinal fluid; <sup>a</sup>All units of measurements are mg/l.

method depending on the frequency of requests (good practice point).

## CSF markers with a future potential to be used in clinical routine

### Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is a glycosylated homodimeric protein of approximately 45 kDa. It is produced by a broad range of cell types in response to stimuli such as hypoxia or tumor necrosis factor (TNF)-alpha [109–113]. VEGF is selectively mitogenic for endothelial cells and plays a fundamental role in both normal and abnormal angiogenesis [114]. VEGF can be measured with several commercially available sandwich enzyme-linked immunosorbent assays. Because those tests use various capture reagents, the resulting 'normal' ranges of VEGF in CSF depend on the test used.

Vascular endothelial growth factor in CSF was suggested to determine prognosis in several malignancies. VEGF was negatively correlated with survival in patients with leptomeningeal metastases [115,116] (class II), and astrocytic brain tumors [117,118] (class III). Furthermore, it serves as a biologic marker for the diagnosis and evaluation of treatment response in leptomeningeal metastases [119] (class III) (Table 4). VEGF is not detectable in lower grade gliomas (grade 2) [120] (class III).

Studies investigating CSF VEGF in a number of other neurological conditions are summarized in Table 5 demonstrating a possible role in the pathogenesis of some diseases as well as some inconsistent findings.

### Recommendations

Elevated VEGF levels (cut-points between 100 and 650 pg/ml) in CSF are highly sensitive (73–100%) and specific (73–100%) for leptomeningeal metastases from solid but not from hematological tumors (level A recommendation) and are associated with a poor prog-

nosis in this condition (level B recommendation). CSF VEGF levels appear to correlate with the grade of malignancy of CNS gliomas (level C recommendation). Normal to elevated as well as decreased VEGF in CSF can be found in various neurological diseases (bacterial meningitis, ALS, POEMS, and SAH) suggesting a possible pathogenic role without relevant diagnostic or prognostic value (level B recommendation).

### CSF neurofilaments

Neurofilaments (Nfs) constitute the axonal cytoskeleton [121]. There are four Nf subunits: a light (NfL, 68 kDa), intermediate (NfM, 115 kDa) and heavy chain (NfH, 190–210 kDa) and also alpha-internexin. NFs are released into the CSF following axonal damage. The normal upper reference ranges for CSF NfH and NfL levels are presented in Table 6.

A comprehensive list of studies on NfL and NfH including cut-points and diagnostic sensitivity and specificity values is summarized in Table 6. Reference values depend on many pre-analytical and analytical factors and vary between laboratories. It is therefore, strongly encouraged that each laboratory establish its own reference values.

Neurofilaments are of prognostic value in subarachnoid hemorrhage (SAH). Increased levels of NfH and NfL in ventricular and lumbar CSF are related to poor outcome using the Glasgow outcome score (class I) [122–125] [126–128]. In MS, high CSF NfL levels were of prognostic value on a number of clinical outcome scales (class III) [129,130]. The heavy chain of Nfs in CSF was also related to progression within the subsequent year in RRMS patients (class III) [131–133]. Moreover, CSF NfH levels in the CSF or plasma of patients with optic neuritis (ON) predicted the degree of permanent loss of visual function (class III) [132,134]. Finally, CSF NfH levels were significantly higher in patients with neuromyelitis optica (NMO) which is consistent with the clinical experience of more severe disease and more extensive axonal loss in NMO than in MS (class III) [135].

**Table 4** Vascular endothelial growth factor diagnostic sensitivity and specificity in leptomeningeal metastases

Sensitivity	Specificity	Cut-point	Method /isoform	Class of evidence	Reference
100% (n = 19)	73%	(log tPA index–0.7229 * log VEGF index) < –0.18182	ELISA/121,165		[205]
85% (n = 53)	100%	> 262 ng/ml	ELISA	II	[115]
73% (n = 37)	93%	> 100 ng/ml	ELISA/121,165	II	[116]
51%	98.3%	> 250 ng/ml			
100% (n = 11)	100%	> 633.1 ng/ml	ELISA/121,165	III	[119]

**Table 5** VEGF levels in studies not investigating leptomeningeal metastases

Study population (N)	VEGF mean <sup>a</sup> values (pg/ml) <sup>a</sup>	Control group	VEGF controls mean values (pg/ml) <sup>a</sup>	Method/ isoforms	Class of evidence	Reference
<b>Meningitis</b>						
Tuberculous (48) <sup>b</sup>	106 <sup>c</sup> –144 <sup>c</sup>	Various other forms of meningitis	5.7–80.1	ELISA/121, 165	II	[206,207]
Eosinophilic (9)	568	None		ELISA/165	IV	[208]
Pneumococcal (10)	Below lod <sup>d</sup>	Viral meningitis (10) non-inflammatory controls (10)	Slightly elevated below lod	Protein array	III	[209]
Cryptococcal (95)	37.5 <sup>c</sup> (geometric mean)	Spinal anesthesia (17)	Below lod	ELISA/121,165	2	[210]
Any bacterial (37)	30% above cut-point (25 pg/ml) <sup>c</sup>	Viral meningitis (16) Non-inflammatory controls (35)	Below lod Below lod	ELISA	III	[211]
HIV with CNS infection (8)	49.7	HIV negative CNS infection (18)	43.7	ELISA	III	[212]
HIV without CNS infection (19)	42.6					
CNS glioma grade 2 (7)	Below lod	Controls unspecified (3)	Below lod	ELISA	III	[120]
CNS glioma grade 1 and 2 (7)	Median 7.2 ng/ml 17.6 ng/ml <sup>c</sup>	Hydrocephalus (10)	Median 8.3 ng/ml	ELISA	Uncertain <sup>e</sup>	[117]
CNS glioma grade 3 and 4 (19)						
CNS glioma grade 3 and 4 (27)	4.9 ng/mg of total protein	Various tumors (31)	0.006 ng/mg of total protein	Not stated	IV	[118]
Amyotrophic lateral sclerosis (105) <sup>b</sup>	Various ways of expressing VEGF levels (pg/ml, pg/l, int. Units)	Various groups including 'neurological' controls, neurodegenerative disorders, headache, radiculopathies, total <i>n</i> = 124	Ranging from significantly lower to equal to higher	ELISA/165	All class III	[213–217]
Pre-eclampsia (15)	6.6	Pregnant normal pressure	5.5	ELISA	III	[218]
Alzheimer disease (43) <sup>b</sup>	Below lod	'Healthy' controls (27)	Below lod	ELISA	III	[219,220]
	500 <sup>c</sup> (only versus vasc. dementia)	Vascular dementia (26)	330	ELISA/165	Uncertain <sup>f</sup>	
		'Healthy' controls (27)	130	ELISA/165		
SAH (14)	90	Non-SAH and 'healthy' controls (14)	13–19	ELISA	III, IV	[221,222]
SAH (15)	0–2000	None		ELISA/165		
POEMS syndrome (10)	6.8	Other neurological disease, GBS, CIDP (total 40)	3.0–9.1	ELISA	III	[223]

VEGF, vascular endothelial growth factor; CNS, central nervous system; <sup>a</sup>Unless stated otherwise; <sup>b</sup>Total number derived from more than one study; <sup>c</sup>significant difference between study population and one or more control groups; <sup>d</sup>lod: limit of detection; <sup>e</sup>uncertain because VEGF concentrations are 1000 times higher than in most other studies; <sup>f</sup>uncertain because of unusually high levels in 'healthy' controls.

In amyotrophic lateral sclerosis (ALS) patients high CSF NfH levels were related to a more rapid clinical progression and with upper motor neuron symptoms (class II) [136,137]. In the AIDS–dementia complex elevated CSF NfL levels were shown to be a good secondary outcome measure in an antiretroviral treatment trial (class III) [138–142]. CSF Nf levels could discriminate different Parkinsonian disorders. NfL and NfH levels were higher in patients with multi-system atrophy compared to patients with Parkinson's disease (class II) [143–145].

A recent meta-analysis reviewing the value of CSF NfL and NfH in neurodegenerative dementia con-

cluded that CSF NfL and NfH levels were increased in AD, frontotemporal lobar dementia (FTLD) and vascular dementia (class III) (see Table 6 for references and [122]). In comparison to CSF tau and A $\beta$ <sub>1–42</sub> levels the diagnostic accuracy of CSF NfL and NfH levels is lower.

#### Recommendations

Cerebrospinalfluid neurofilament levels can be used as a biomarker for neuronal death and axonal degeneration (level B rating). The prognostic value of CSF Nf levels is highest in acute conditions such as SAH, ALS, MSA, MS, acute ON and NMO (level B rating). There is

Table 6 Conditions in which increased NF has been shown

Condition	NF-H	Remarks	Sensitivity	Specificity	NF-L	Remarks	Sensitivity	Specificity	Level of evidence
Cut-off value	0.73 ng/ml [122]	Based on a reference population from a neurological hospital ( <i>n</i> = 416)			Rosengren: 125 pg/l; < 60 years: < 0.25 ng/ml; 60–70: 0.38 ng/ml; 71–80: < 0.75 ng/ml; Norgren: 100 ng/l. Van Geel: 40 ng/l	Population based reference values (healthy volunteers)			
Condition in which neurofilament is increased									
ALS	[122,135,137]	Five times higher than in controls. In upper motor symptoms two times higher than in lower motor symptoms	At cut-off of 0.73: 80% for ALS versus control. ALS versus AD: 80	75% (ALS versus control) ALS versus AD: 97%	[127,136,224]	About 10 times increase in patients with upper motor symptoms. About two times increase in patients with lower motor symptoms	n.a.	n.a.	Class II
PD & MSA	[143,145]	About 3 times elevated in MSA, not elevated in IPD	NfH: cut-off of 114.5: 83% for MSA-P versus IPD 77% for PSP from IPD (cut-off of 1.4 ng/ml)	87% for MSA versus IPD; 94% for PSP versus IPD	[144,145]	About seven times elevated in MSA, not elevated in IPD	NF-L: cut-off of 17.15: 83% for MSA-P versus IPD	90%	Class III
PSP	[143]	The ROC optimised cut-off level of 1.48 ng/ml is used for distinguishing patients with PSP from those with PD or CBD	76.5%	93.8%	[144]	n.a.	n.a.	n.a.	Class III
MS	In most MS patients CSF NfH levels are within the normal reference range [122].	At a cut-off: 160 pg/ml: sensitivity 34% and specificity 88% for conversion from CIS to RRMS [170]. Contradictory results found by Lim [134]. Relation with progression. Phosphorylation rate eight times higher in severely disabled patients compared to mildly disabled patients [132] and also prognostic for deterioration at follow-up [131]. NfH increases during follow-up [121]	n.a.	n.a.	[127,129,130]	Not increased: [133]	78% for MS; 91% for relapse, 44% for remission, 48% for SP	92–100%	Relation with progression: class II

Table 6 (Continued)

Condition	NF-H	Remarks	Sensitivity	Specificity	NF-L	Remarks	Sensitivity	Specificity	Level of evidence
AD	[225,226]	Using ROC optimised cut-off levels. NfH higher in AD compared to controls. Effect size 0.71 [227]	[225] 57.5%	77.0%	[127,228-230]	Increased in AD and VD compared to controls. In the meta-analysis the effect size distinguishing AD from controls was 1.27 with AD patients having the higher CSF NfL levels [227]	58-78%	28-77%	Class II
FTLD	[226,230]	NfH higher in FTLD compared to controls. Effect size 0.74 [227]			[226,228, 230-232]	NfL Higher in FTLD compared to controls. Effect size was 1.38 [227]	82% to discriminate FTD from EAD in [226] contradictory with Pijnenburg <i>et al.</i>	70%	Class II
VD	[225]	NfH is higher in VD then in AD	n.a.	n.a.	[127,228, 229,233]	NfL Higher than in AD then in controls. Effect size 1.24 [227]	85%	68%	Class II
DLBD	[226]	Using ROC optimised cut-off levels. NfH higher in DLBD compared to late AD	89%	28%		Using ROC optimised cut-off levels. NfH higher in DLBD compared to late AD	33%	82%	Class III
Spinal cord injury	n.d.				[234]	5 to 450x increased in acute cervical spine injury, and levels increase during 21 days after the injury	Cut-off of 125 ng/l, 100% sens for acute spinal cord injury, 18% for whiplash	100%	Class III
AIDS-dementia complex	n.d.				[138-142]	<i>n</i> = 8, three subjects increased NF-L after interruption. No clinical signs, but f.u. time only 101 days. Subclinical marker?	n.a.	86% [139]	Class II
SAH	[123-125]	Prognostic for outcome	Cut-off: highest value in survivors: 57-100% for unfavorable outcome	71-75%	[126-128]	Lumbar CSF	Cut-off for unfavorable outcome: 6,400 ng/l; 100% sens	50%	Class I

Table 6 (Continued)

Condition	NF-H	Remarks	Sensitivity	Specificity	NF-L	Remarks	Sensitivity	Specificity	Level of evidence
NPH	n.d.				[196,235–237]	Ten times increased compared to reference values	n.a.	n.a.	Class III
Binswanger disease	n.d.				[235]	Four times increased compared to reference values.	n.a.	n.a.	Class III
GBS	[238]	A prognostic marker for poor outcome. Not a diagnostic test	n.a.	n.a.	n.d.				Class III
Cardiac arrest	n.d.				[239]	Prognostic for axonal damage after cardiac arrest	Poor outcome (Glasgow Coma Scale): 75–92%	80–100% for different cut-offs	Class III
HSV encephalitis relapse	n.d.				[240]	Higher during encephalitis relapse	n.a.	n.a.	Class III

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CBD, cortico-basal degeneration; DLB, diffuse Lewy body disease; FTLD, fronto-temporal lobar degeneration; GBS, Guillain-Barré syndrome; ICH, intracerebral hemorrhage; MMC, meningo-myelocele; MS, multiple sclerosis; MSA, multiple system atrophy; NMO, neuromyelitis optica; ON, optic neuritis; PD, Parkinson's disease; PSP, progressive supranuclear palsy; SAH, subarachnoid hemorrhage; n.a., not available; n.d., not done.

further evidence from longitudinal studies that there is potential for CSF Nf to be used to monitor disease progression in SAH, MS and spinal cord injury (level B rating). Because reference values depend on many pre-analytical and analytical factors, laboratories should establish their own reference values.

### Myelin basic protein

Myelin basic protein (MBP) is a major protein in CNS myelin, but constitutes a lesser proportion of myelin in the PNS [146]. The CSF concentration of MBP increases with age (class I) [147], and many disease processes, even diseases affecting mainly peripheral nerve myelin, may show increases in the CSF concentration of MBP (class II) [148–151]. It is mostly measured by radioimmunoassay, but ELISA assays have also been developed. The CSF concentration of MBP is increased in multiple sclerosis (MS) (class I), where it is higher in relapses than in progression (class I), correlates with the severity of the relapse (class II), is higher in patients with multifocal relapses than in monofocal relapses (class III), and is higher in relapses with new symptoms than with the recurrence of previous symptoms (class III) [152,153–159]. Increased CSF concentrations of MBP in patients in clinical remission may be associated with an increased relapse risk and a lower risk of a benign disease course (class III) [160,161].

The CSF concentration of MBP decreases in parallel with spontaneous remission of clinical symptoms and with the resolution of active brain lesions on Gd-enhanced MRI after treatment with methylprednisolone (class III) [162,163]. Increased CSF concentrations of MBP may indicate a better short-term response to treatment with methylprednisolone, but Gd-enhanced MRI studies are superior to MBP measurements in this respect (class III) [164,165].

### Recommendations

Myelin basic protein immunoassays are technically demanding because the analyte exists both in free and lipid-bound forms and bound in immune complexes. This is often not considered in CSF research. Increased CSF concentrations of MBP are unspecific and can be found in a variety of diseases, not only in MS (level A rating). It is possible that MBP measurements could be of prognostic value in MS (level C rating).

### Recommendations for future research

In general, disease-specific biomarkers for diagnostic, prognostic, and monitoring purposes should be validated using established protocols [166,167]. Furthermore, validation across laboratories is urgently needed

for many biomarkers, as shown for A $\beta$  and Tau proteins [168]. With respect to A $\beta$ 1-42, tau and P-tau, there is a need for evaluation of the precise predictive value of these methods in the differentiation between different neurodegenerative diseases, rather than as for the discrimination between healthy aging and patients with AD (incipient or definite). There seems to be a potential for assays recognizing different phosphoforms of tau in differential diagnosis, and this issue should be pursued in future studies.

Studies of 14-3-3 protein, A $\beta$ 1-42, tau and P-tau may also be helpful as diagnostic aids in patients with suspected CJD, but since CJD is a rare disorder, a very high diagnostic specificity against a wide spectrum of other diseases presenting with rapidly progressing dementia is necessary, and this issue needs to be investigated more thoroughly. The value of measuring hypocretin-1 (orexin-1) levels in CSF of other sleep disorders needs to be addressed. A serum marker for narcolepsy with cataplexy would be helpful for early diagnosis and serial analysis during the course and treatment of the disease.

The diagnostic and prognostic value of VEGF in leptomeningeal metastases should be confirmed in a larger prospective study, specifically in cytology-negative patients. For patients with leptomeningeal metastases from hematological tumors more sensitive markers need to be explored. Regarding the Nf assays, standardization of the assays and variation amongst different laboratories should be explored, in order to define reliable reference values. The use of Nfs in predicting neurological decline must be explored further. Further research should also focus on combining Nfs with other markers, as they will probably not be able to predict neurological decline with sufficient specificity and sensitivity as a stand alone marker.

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For a full list of References, please see Reference Appendix pp. e156–e163.

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