PAPER

CSF neurofilament proteins in the differential diagnosis of dementia

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Background: Neurofilament (NF) proteins are major cytoskeletal constituents of neurons. Increased CSF NF levels may reflect neuronal degeneration.
Objective: To investigate the diagnostic value of CSF NF analysis to discriminate in relatively young dementia patients between frontotemporal lobe degeneration (FTLD) and early onset Alzheimer's disease (EAD; onset $\leqslant$65 years of age), and in elderly dementia patients between dementia with Lewy bodies (DLB) and late onset AD (LAD; onset >65 years of age).
Methods: In CSF of 28 FTLD, 37 EAD, 18 DLB and 33 LAD patients, and 26 control subjects, we analysed NF light chain (NFL), phosphorylated NF heavy chain (pNFH), amyloid β_{42} protein ($A\beta_{42}$), total tau and tau phosphorylated at threonine 181 (p-tau_{181}).
Results: CSF NFL levels were higher in FTLD patients compared with EAD patients ($p<0.001$), and diagnostic accuracy of p-tau_{181} and $A\beta_{42}$ analysis improved with addition of NFL analysis (sensitivity 86%, specificity 100%). CSF pNFH levels were elevated in DLB, LAD and FTLD compared with controls ($p<0.05$) but no significant differences were found between the dementia groups.
Conclusions: In the diagnostic workup of relatively young dementia patients, CSF NFL levels may play a role in the discrimination between FTLD and EAD, especially in combination with $A\beta_{42}$ and p-tau_{181} analysis.

The clinical differentiation between Alzheimer's disease (AD), frontotemporal lobar degeneration (FTLD) and dementia with Lewy bodies (DLB) can be achieved using a combination of clinical criteria, neuroimaging and CSF biomarkers, in particular amyloid β_{42} protein ($A\beta_{42}$), total tau (t-tau) and phosphorylated tau (p-tau).[1] However, in younger patients with incipient or mild dementia, it is often still difficult to discriminate between AD and FTLD. Especially so, as in patients with early onset AD (EAD), focal cortical symptoms (language, praxis or executive function problems) and behavioural deficits can be more prominent than memory dysfunction.[2] A similar challenge exists in older patients for the differentiation between AD and DLB, as neuropsychiatric symptoms and extrapyramidal signs are commonly seen in AD patients with more advanced disease.[3]

Neurofilament (NF) proteins are major constituents of the neuronal cytoskeleton. Localised in large neurons and myelinated axons, they play an important role in neuronal structure. NFs consist of three polypeptides; the light (NFL), medium (NFM) and heavy (NFH) subunits.[4] Increased levels of NFs in CSF may reflect neuronal degeneration in neurological disease.

Analyses of NFs in CSF of dementia patients challenged various researchers. CSF NFL levels were increased in AD, late onset AD (LAD) and FTLD compared with controls, and tended to be increased in FTLD compared with EAD.[5–10] A positive correlation between CSF NFL levels and the degree of cognitive impairment was found in FTLD and LAD.[9]

Less is known about CSF levels of the other NF subunits. One study described increased CSF levels of phosphorylated NFH/M in AD compared with vascular dementia (VaD) and controls.[5] Others found elevated CSF NFH levels in AD and VaD compared with controls, but no differences between FTLD and controls, or between AD, VaD and FTLD.[11]

We assumed that widespread neuronal degeneration leads to elevated NF levels in CSF, and therefore studied CSF levels of NFL and phosphorylated NFH (pNFH) in patients with neurodegenerative dementias. We investigated whether CSF NF protein analysis helps to discriminate between FTLD and EAD in relatively young dementia patients, and between DLB and LAD in older dementia patients, and whether it has superior or additional diagnostic value compared with $A\beta_{42}$, t-tau, and p-tau analysis.

METHODS

Patients

This retrospective study included 37 EAD (onset before or at 65 years of age), 33 LAD (onset after 65 years of age), 18 DLB and 28 FTLD patients, identified through the CSF databases of the Radboud University Nijmegen Medical Centre, and the VU Medical Centre, Amsterdam, the Netherlands. Only patients with a probable diagnosis according to the accepted clinical diagnostic criteria were included.[12–14] The standard diagnostic examination protocol included medical history, physical and neurological examination, neuropsychological testing, laboratory testing, brain imaging and a lumbar puncture.

Twenty-six control subjects were included who underwent a lumbar puncture for various reasons, but did not have a neurological disorder.

CSF analysis

Lumbar punctures were performed after informed consent was obtained from the patient or the legal representative. CSF was collected in polypropylene tubes, transported to the adjacent laboratory within 30 min, centrifuged after routine

Abbreviations: $A\beta_{42}$, amyloid β_{42} protein; AD, Alzheimer's disease; AUC, area under the curve; DLB, dementia with Lewy bodies; EAD, early onset Alzheimer's disease; FTLD, frontotemporal lobe degeneration; LAD, late onset Alzheimer's disease; NF, neurofilament; NFL, neurofilament light chain; NFM, neurofilament medium chain; pNFH, phosphorylated neurofilament heavy chain; p-tau_{181}, tau phosphorylated at threonine 181; t-tau, total tau; VaD, vascular dementia

Table 1 Clinical characteristics and levels of CSF markers

Parameter	EAD	FTLD	LAD	DLB	Controls
Age (y)	61 (52–69)	63 (43–79)	76 (69–90)	72 (58–90)	60 (53–85)
No of patients (M/F)	37 (15/22)	28 (20/8)	33 (13/20)	18 (13/5)	26 (12/14)
Disease duration (y)	3.0 (1.0–10.0)	3.0 (1.0–10.0)	2.0 (0.5–7.0)	1.5 (1.0–5.0)	
MMSE score	20 (6–28)	24 (3–28)	21 (9–27)	23 (2–28)	
NFL (pg/ml)	6.1 (n = 37)* (0.0–40.3)	16.9 (n = 28)††† ‡‡(0.0–76.4)	15.2 (n = 33) (0.0–70.1)	10.4 (n = 18) (0.0–60.4)	5.0 (n = 26) (0.0–33.8)
pNFH (pg/ml)	88 (n = 36)¶ (39–205)	109 (n = 28)† (52–373)	124 (n = 29)† (49–398)	131 (n = 18)††† ‡‡ (71–711)	84 (n = 24) (38–112)
$A\beta_{42}$ (pg/ml)	365 (n = 37)** (184–703)	582 (n = 28)¶ ‡‡‡ §§§ (202–1408)	419 (n = 33)** (197–873)	444 (n = 18)* (176–784)	–
t-tau (pg/ml)	565 (n = 37)* ¶ (173–1946)	362 (n = 28)‡‡ §§ (115–983)	647 (n = 33)* ¶¶ (178–2400)	270 (n = 18)‡‡ §§ (105–961)	–
p-tau_{181} (pg/ml)	86 (n = 35)** ¶¶ (47–250)	51 (n = 28)‡‡‡ §§§ (24–132)	89 (n = 33)** ¶¶ (31–254)	58 (n = 17)‡‡‡ §§§ (32–89)	–

$A\beta_{42}$, amyloid β_{42} protein; DLB, dementia with Lewy bodies; EAD, early onset Alzheimer's disease; FTLD, frontotemporal lobar degeneration; LAD, late onset Alzheimer's disease; MMSE, Mini-Mental State Examination; NFL, neurofilament light chain; pNFH, phosphorylated neurofilament heavy chain; p-tau_{181}, tau phosphorylated at threonine 181; t-tau, total tau.
Values are expressed as medians (range).
*p<0.01, **p<0.001 compared with FTLD.
†p<0.05, †††p<0.001 compared with controls.
‡‡p<0.01, ‡‡‡p<0.001 compared with EAD.
¶p<0.01 , ¶¶p<0.001 compared with DLB.
§§p<0.01, §§§p<0.001 compared with LAD.

investigations and immediately aliquoted and stored at −80℃ until analysis.

Determination of NFL levels was performed using our previously described sandwich ELISA.[15] Levels of pNFH were determined using a modified version of a sandwich ELISA developed previously[16] that we recently described in more detail.[17] T-tau, $A\beta_{42}$ and p-tau_{181} were measured using ELISA (Innogenetics NV, Gent, Belgium).

In two controls, three EAD, four LAD and one DLB patient, the CSF amount was insufficient to measure either pNFH or p-tau_{181}.

Statistical analysis

CSF NFL and pNFH levels followed a log normal distribution, and therefore the statistical analysis was carried out on log transformed values. Tukey's method for multiple comparisons was used for group comparisons. In additional analyses, age and gender were included as covariates. For each CSF marker, the area under the receiver operating characteristic curve (AUC), cut-off values, sensitivity and specificity were calculated. Logistic regression with backwards selection was used to derive combinations of CSF markers with the highest diagnostic value. For correlations, Spearman's rank coefficient was used.

RESULTS

Patient characteristics are shown in table 1. Our main study groups were matched for age. Median age of controls was significantly lower compared with LAD and DLB patients. Both age and gender were included in the statistical analysis, but did not substantially change the results.

CSF NFL levels were significantly higher in FTLD compared with EAD and controls, but were comparable in DLB and LAD (table 1). CSF pNFH levels were significantly elevated in LAD, FTLD and DLB compared with controls (table 1). Furthermore, CSF pNFH levels were significantly higher in DLB than in EAD, but no differences were found between DLB and LAD, or between FTLD and EAD. In none of the dementia groups or dementia patients as a whole, was a significant correlation between CSF NFL or pNFH and MMSE score, disease duration or age found.

In FTLD compared with EAD, CSF $A\beta_{42}$ was significantly higher, and t-tau and p-tau_{181} lower (table 1). CSF $A\beta_{42}$ levels were comparable in DLB and LAD. CSF levels of t-tau and p-tau_{181} in DLB were significantly lower compared with LAD.

When discriminating between FTLD and EAD using CSF NFL levels, sensitivity was 82% and specificity 70% (table 2; AUC = 0.80). This discriminative value was comparable with $A\beta_{42}$ and p-tau_{181}. However, a combination of CSF levels of

Table 2 Discriminative value of CSF markers between patient groups

	FTLD vs EAD				DLB vs LAD			
CSF markers	AUC	Cut off (pg/ml)	Sens (%)	Spec (%)	AUC	Cut off (pg/ml)	Sens (%)	Spec (%)
NFL	0.80	6.7	82	70	0.53	6.0	33	82
pNFH	0.62	129	46	78	0.55	84.8	89	28
$A\beta_{42}$	0.78	488	64	92	0.49	590	89	21
t-tau	0.71	420	68	70	0.82	361	72	88
p-tau_{181}	0.81	53.0	57	97	0.86	68.7	82	85
$A\beta_{42}$+p-tau_{181}	0.89*	−8.1†	75	94	0.88	22.5§	82	94
$A\beta_{42}$+p-tau_{181}+NFL	0.92**	−4.1‡	86	100	0.88	23.0¶	82	94

$A\beta_{42}$, amyloid β_{42} protein; AUC, area under the curve; DLB, dementia with Lewy bodies; EAD, early onset Alzheimer's disease; FTLD, frontotemporal lobar degeneration; LAD, late onset Alzheimer's disease; NFL, neurofilament light chain; pNFH, phosphorylated neurofilament heavy chain; p-tau_{181}, tau phosphorylated at threonine 181; Sens, sensitivity; Spec, specificity ; t-tau, total tau.
*Statistically significant improvement versus p-tau_{181} alone; **statistically significant improvement versus p-tau_{181} and $A\beta_{42}$ combination.
The discriminant formulas for the combined markers are:
†2.6*ln(ptau_{181})−3.0*ln($A\beta_{42}$)
‡3.5*ln(ptau_{181})−2.3*ln($A\beta_{42}$)-2.0*ln(NFL)
§3.8*ln(ptau_{181})+1.0*ln($A\beta_{42}$)
¶3.9*ln(ptau_{181})+1.0*ln($A\beta_{42}$)+0.1*ln(NFL).

$A\beta_{42}$ and p-tau_{181} improved sensitivity and specificity significantly compared with p-tau_{181} alone (AUC = 0.89), and even more so when CSF NFL levels were added (AUC = 0.92). CSF NFH levels did not offer additional diagnostic value.

When differentiating DLB from LAD, the combination of CSF p-tau_{181} and $A\beta_{42}$ performed best (table 2). CSF NFL or pNFH measurements did not have additional discriminative value.

DISCUSSION

In FTLD, CSF NFL levels were increased, consistent with other studies,[6 9 10] and the analysis had additional diagnostic value in the differentiation of FTLD from EAD, in combination with p-tau_{181} and $A\beta_{42}$. In contrast with a recent study, we also found elevated CSF pNFH levels in FTLD.[11] Thus, perhaps cytoskeleton proteins other than tau are involved in the pathophysiology of FTLD.[9 10] The heterogeneity in the underlying pathology of FTLD, however, makes it difficult to disentangle the exact function of NFs in this process.[18] It has been hypothesised that NFs are either defective or overexpressed in FTLD and aggregate intracellularly, disrupting the cytoskeleton and cell integrity, causing cytoskeleton protein leakage into the CSF, and premature cell death.[9] As only CSF levels of NFL and pNFH, but not tau, are increased, this suggests that NFs are overexpressed in neurons, and that these neurons are selectively vulnerable in FTLD.

In DLB compared with LAD, CSF levels of NFL and pNFH had no discriminative value. Nevertheless, our observation of elevated pNFH levels in DLB contributes to the emerging picture of abnormal CSF protein composition in degenerative dementias. As CSF NFL levels were not increased in DLB, the difference in CSF NF and pNFH composition compared with FTLD suggests differences in the underlying pathophysiological mechanism of these disorders. There is evidence that phosphorylated and non-phosphorylated NFs accumulate in Lewy bodies. Also, an increased number of cortical NF containing neurons was observed in DLB compared with AD and controls,[19] which apparently does not lead to increased CSF NFL concentrations. In sum, this suggests that in DLB, AD and FTLD, different cortical neuronal populations are affected, and different subsets of NFs are involved.

In LAD, we observed elevated CSF pNFH levels, corroborating earlier observations.[11] We could not confirm previous reports that showed increased CSF NFL levels in AD.[5–9 20] Surprisingly, a non-significant trend of increased CSF NFL and pNFH levels in LAD compared with EAD was found, consistent with another study describing elevated CSF NFL levels in LAD compared with EAD.[9] As an association between the presence of white matter changes and increased CSF NFL was previously described,[8] and white matter lesions are more common in LAD than EAD patients, this might explain our observation.

The implications of our study may be limited because of the relatively small numbers of patients. This, together with the absence of post-mortem verification, might explain the considerable dispersion in CSF NFL and pNFH levels found in each patient group. Nevertheless, compared with previous studies on NFs in CSF, our FTLD group is the largest, and our DLB group is the first ever described. Also, the absence of post-mortem verification applies to many studies on biomarkers in dementia. To compensate for the lack of post-mortem diagnoses, we conducted an extensive diagnostic examination protocol.

We conclude that measurement of CSF NFL levels can play a role in the diagnostic workup of patients with FTLD and EAD, particularly in combination with $A\beta_{42}$ and p-tau_{181} analysis. The contribution of CSF NFL analysis to the clinical management of these relatively young dementia patients will have to be established through larger prospective randomised masked validation studies. In addition, ongoing research may shed more light on pathophysiological mechanisms explaining the observed elevated CSF levels of NFL in FTLD, and pNFH in FTLD, LAD and DLB.

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