Short communication

Cerebrospinal fluid neurofilament light in suspected sporadic Creutzfeldt-Jakob disease

Eirini Kanata [a,*], Ewa Golanska [b], Anna Villar-Piqué [c], Aikaterini Karsanidou [a], Dimitra Dafou [d], Konstantinos Xanthopoulos [a], Matthias Schmitz [c,e], Isidro Ferrer [f,g], André Karch [h], Beata Sikorska [b], Pawel P. Liberski [b], Theodoros Sklaviadis [a], Inga Zerr [c,e,1], Franc Llorens [c,f,i,1,*]

[a] Laboratory of Pharmacology, School of Health Sciences, Department of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece
[b] Department of Molecular Pathology and Neuropathology, Medical University of Lodz, Lodz, Poland
[c] Department of Neurology, University Medical School, Göttingen, Germany
[d] Department of Genetics, Development, and Molecular Biology, School of Biology, Aristotle University, Thessaloniki, Greece
[e] German Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany
[f] Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED), Institute Carlos III, Ministry of Health, L'Hospitalet de Llobregat, Spain
[g] Bellvitge University Hospital-IDIBELL, Department of Pathology and Experimental Therapeutics, University of Barcelona, Hospitalet de Llobregat, Spain
[h] Department of Epidemiology, Helmholtz Centre for Infection Research, Braunschweig, Germany
[i] Bellvitge Biomedical Research Institute (IDBELL), L'Hospitalet de Llobregat, Spain

ARTICLE INFO

ABSTRACT

Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form of human prion disease. It is invariably fatal and displays a short clinical disease stage. The key event in sCJD is the propagation of a beta-sheet rich conformer of the physiological PrP^C protein, known as PrP^{Sc}. Neuropathological disease characteristics include gliosis, neuronal loss and spongiform degeneration; disease clinical manifestations refer to mental and visual disabilities, cognitive impairment, gait or limb ataxia, myoclonus and mutism. Definite sCJD diagnosis requires post-mortem brain material histopathological examination. However, highly certain pre-mortem differential diagnosis is desired to exclude other treatable disorders and to reduce disease transmission risks. Detection and/or quantification of cerebrospinal fluid (CSF) biomarkers reflecting neuronal damage and PrP^C misfolding in the diseased brain significantly enhance pre-mortem diagnosis. Previously established and newly identified biomarkers are used towards this direction. Increased CSF Neurofilament light chain (NFL) concentrations have been reported in several neurological disorders, including prion diseases. In the present study, we analyzed CSF NFL levels in two independent patient cohorts, consisting of highly suspected sCJD cases that were further classified as sCJD or non-CJD according to established diagnostic criteria. CSF NFL concentrations were increased in sCJD compared to non-CJD cases in both cohorts (area under the curve (with 95% confidence interval) equal to 0.89 (0.82 to 0.97) and 0.86 (0.77 to 0.96), respectively. CSF NFL was associated neither to age nor to sex but correlated with total-tau concentrations in both cohorts. Overall, our data provide independent validation of CSF NFL utility in sCJD differential diagnosis.

1. Introduction

Neurofilament light chain (NFL) corresponds to the small subunit of the neuronal intermediate filament heteropolymers, the main components of the neuronal cytoskeleton. NFL is mainly detected in large myelinated axons, providing structural stability to the axonal caliber and facilitating conduction velocity [1]. Recently, NFL, has been detected in the post-synaptic area and found to affect synaptic plasticity [2,3]. NFL enrichment in axons and its presence in synapses suggest that axonal injury and synaptic/neuronal death occurring in several

neurodegenerative/neurological disorders may result in increased cerebrospinal fluid (CSF) NFL concentrations. Thus, increased body fluid NFL levels have been suggested to indicate neuroaxonal and white matter degeneration [4,5].

The diagnostic potential of CSF NFL has been studied in the context of several neurodegenerative disorders, including Alzheimer's disease (AD) [6], Amyotrophic Lateral Sclerosis (ALS) [7–9], MS [10] and their related disorders, corresponding to Mild Cognitive Impairment (MCI) [6] and different Frontotemporal Dementia subtypes (FTDs) [11–13]. Further, a potential prognostic value in terms of disease progression and survival has been suggested for CSF NFL levels in ALS [7], and in FTD subtypes [12,14].

Similar to other neurodegenerative disorders, increased CSF NFL levels have been reported in sporadic Creutzfeldt-Jakob disease (sCJD) [15], the most prevalent human prion disease, characterized by rapidly progressive dementia and a short disease duration. The diagnostic accuracy of CSF NFL in the differential diagnosis of prion diseases has been recently assessed, showing highest concentrations in sCJD compared to other neurodegenerative disorders (AD, Lewy body dementia, FTD, vascular dementia) and to MCI cases [16].

The aim of the present study was to further assess the utility of CSF NFL in the differential diagnosis of sCJD in two independent cohorts comprised of highly suspected sCJD cases. Our data are in line with former studies and provide independent validation of CSF NFL diagnostic potential in sCJD.

2. Materials and methods

2.1. Study population

Cohort 1 was collected at Polish neurologic and psychiatric hospital departments and processed at the Department of Molecular Pathology and Neuropathology (Medical University of Lodz-

Poland). Cohort 2 was collected at the Neurologic Clinics of Northern Greece hospitals and processed at the Laboratory of Pharmacology, School of Pharmacy-Aristotle University of Thessaloniki-Greece. The non-CJD group was composed of subjects with cognitive impairment or dementia (of unknown aetiology) initially suspected of CJD, in which CJD was subsequently excluded. All sCJD patients were classified as probable or definite cases according to diagnostic consensus criteria [17–19].

2.2. CSF tests

CSF NFL and total tau (tau) were quantified using the NFL (Uman-Diagnostics) and INNOTEST®hTAU-Ag (Fujirebio) ELISA assays, respectively. CSF was analyzed for the presence of 14-3-3 protein as described before [20].

2.3. Statistical tests

Mann-Whitney U tests were used to compare two groups of samples. To assess diagnostic accuracy, receiver operating characteristic (ROC) curve analyses were performed and areas under the curve (AUC) with 95% confidence intervals (95% CI) calculated. Comparison between AUCs was performed using the DeLong's test [21], available in the R package pROC [22]. The best cutoff value was estimated based on Youden Index derived from the cohort 2 (training cohort); the diagnostic accuracy (sensitivity and specificity) of NFL in all cases was externally validated in the cohort 1 (validation cohort).

3. Results

The cohorts analyzed in this study were sex and age-matched. Increased tau concentrations and the presence of 14-3-3 protein

	non-CJD	sCJD		non-CJD	sCJD
n	34	43		29	21
age (years)	63 ± 10	64 ± 9		63 ± 13	66 ± 13
gender (f/m)	19/15	21/22		15/14	11/10
tau (pg/mL)	599 ± 375	3918 ± 3009 ***		434 ± 286	6359 ± 3801 ***
14-3-3 (P/T/N)	3/2/29	40/1/2 ***		2/1/26	20/0/1 ***
NFL (pg/mL)	5404 ± 6324	19620 ± 15000 ***		4453 ± 4637	21820 ± 18824 ***

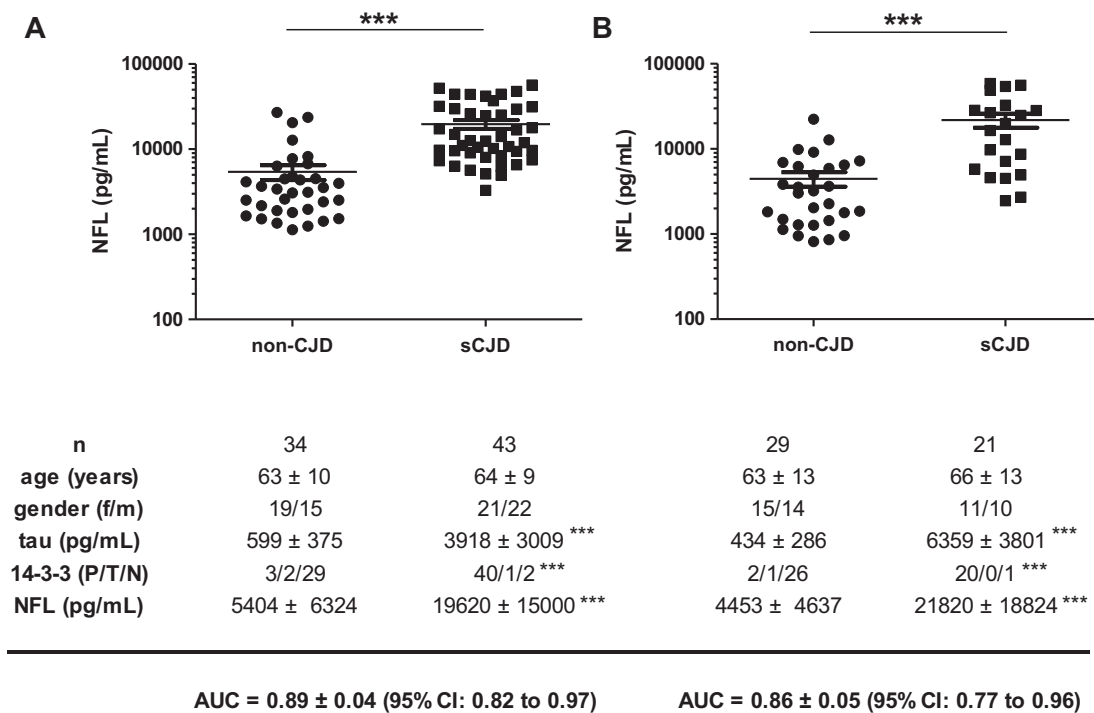AUC = 0.89 ± 0.04 (95% CI: 0.82 to 0.97)   AUC = 0.86 ± 0.05 (95% CI: 0.77 to 0.96)

Fig. 1. Diagnostic value of CSF NFL as a discriminatory biomarker of sCJD from non-prion cases with cognitive impairment/dementia. NFL concentrations in non-CJD and sCJD cases in two independent cohorts (A, cohort 1, Poland and B, cohort 2, Greece). The non-CJD group was composed of subjects with cognitive impairment or dementia (of unknown aetiology) suspected of CJD at a preliminary stage of diagnosis. sCJD cases had probable or confirmed diagnosis. Mann-Whitney U tests were used. NFL levels were significantly different between non-CJD and sCJD (p < 0.001) in both cohorts. Demographic data, biomarker outcomes, as well as Area Under the Curve (AUC) with 95% Confidence Interval (CI) are indicated. 14-3-3 cases were classified as positive (P), trace (T) or negative (N). For statistical analysis, 14-3-3 trace cases were considered as negative.

were detected in the CSF of sCJD cases in both cohorts (p < 0.001), in agreement with previously reported alterations observed in similar clinical groups [23,24] (Fig. 1).

In both cohorts, CSF NFL concentrations were similar among the same diagnostic groups and significantly higher in the sCJD group compared to non-CJD (p < 0.001). A mean concentration of 19620 ± 15000 pg/mL and of 21820 ± 18824 pg/mL in sCJD patients was determined in cohort 1 and cohort 2 respectively (Fig. 1).

The diagnostic accuracy of CSF NFL for each patient cohort was estimated based on ROC curve analysis and subsequent AUC value determination with a 95% CI. Similar AUC values, presenting no statistically significant differences (p = 0.5429), were calculated for both cohorts (cohort 1: AUC: 0.89, 95% CI: 0.82–0.97 and cohort 2: AUC: 0.86, 95% CI: 0.77–0.96).

A cutoff of 4200 pg/mL NFL determined in the cohort 2 (training cohort) revealed 98% sensitivity and 65% specificity in the discrimination of sCJD from non-CJD cases when applied to cohort 1 (validation cohort).

Additionally, a positive association between tau and NFL concentrations in sCJD cases was observed in both cohorts (r = 0.30, p = 0.049 in cohort 1 and r = 0.43, p = 0.046 in cohort 2).

4. Discussion

This study aimed at the further assessment of CSF NFL utility in the differential diagnosis of sCJD. For this purpose we used two independent patient cohorts, consisting of initially highly suspected sCJD cases, further classified as non-CJD or sCJD cases according to consensus diagnostic criteria. This approach enabled the assessment of CSF NFL diagnostic value for the differential sCJD diagnosis within the clinical practice context.

Even though final diagnosis was not available for non-CJD cases, reflecting a limitation of our study, cognitive impairment or dementia were common clinical signs in the non-CJD patients groups. In this regard, the CSF NFL AUC values determined in this study are in line with those reported by others (AUC = 0.9) following comparison of sCJD to a group of neurodegenerative dementias of different aetiologies and to MCI cases utilizing independent cohorts [16]. Altogether our results validate (i) the increased CSF NFL levels in sCJD cases, previously reported by other studies [15,16,25–27] and, (ii) the accuracy of CSF NFL in the discrimination of sCJD from non-prion cases that were considered as highly suspected for sCJD at initial clinical evaluation.

Our data further indicate that the CSF NFL diagnostic accuracy for sCJD discrimination is limited compared to other validated CSF biomarkers for prion diseases diagnosis, such as the real-time quaking induced conversion (RT-QuIC) assay, the p-tau/tau ratio and α-synuclein [23,28–31]. However, in these studies the diagnostic accuracies were calculated using either healthy/neurological controls or neurodegenerative diseases that may not be clinically presenting as sCJD. Thus, the hereby presented diagnostic accuracy of CSF NFL in discriminating sCJD cases, validated in two cohorts of suspected sCJD cases, would be closer to clinical reality. In this regard, our results show that NFL is able to detect with high accuracy sCJD cases (95% sensitivity) using a cut off value significantly lower than those previously reported at the expense of a low specificity (65% specificity).

An important aspect in the field of neurodegeneration is the search for biomarkers that could be utilized for the evaluation of the efficacy of disease modifying therapies in clinical trials [32]. Such biomarkers should ideally cover the full spectra of disease related pathological features. In prion diseases, tau and α-synuclein are considered surrogate markers of neuro-axonal and synaptic damage, respectively, while RT-QuIC represents a direct prion diseases marker. In this regard, the possible primary white matter involvement in sCJD pathology, previously reported based on magnetic resonance imaging data [33,34], could be further assessed exploiting CSF NFL as a new marker that reflects white matter alterations and axonal damage. The positive correlation between CSF NFL and tau concentrations observed in sCJD cases supports this notion. However, since NFL has been also reported in synaptic densities, it is tempting to speculate that CSF NFL is reflecting both white matter degeneration and synaptic loss, although its precise contribution corresponding to neuronal and/or white matter alterations in different pathological conditions cannot be delineated. Further studies correlating neuroimaging and CSF NFL data are expected to shed more light in the assessment of NFL as a white matter damage surrogate marker in sCJD.

Authors' contributions

EK, FL and IZ designed the study. EK, AV-P, AiK and FL performed experiments. EK, EG, AV-P, AK and FL analyzed data. EG, DD, KX, MS, IF, AK, BS, PPL, TS and IZ contributed to contributed to clinical data acquisition, interpretation, and sampling. EK and FL drafted the manuscript.

Ethics approval and consent to participate

The study was conducted according to the revised Declaration of Helsinki and Good Clinical Practice guidelines and was approved by local Ethics committees. Informed consent was given by all study participants or their legal representative.

Competing interests

The authors declare that they have no competing interests.

References

[1] Yuan A, Rao MV, Veeranna Nixon RA. Neurofilaments and neurofilament proteins in health and disease. Cold Spring Harb Perspect Biol 2017;9(4).

[2] Yuan A, Sershen H, Veeranna, et al. Neurofilament subunits are integral components of synapses and modulate neurotransmission and behavior in vivo [Internet]. Mol Psychiatry 2015;20(8):986–94. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4514553&tool=pmcentrez&rendertype=abstract.

[3] Yuan A, Nixon RA. Specialized roles of neurofilament proteins in synapses: relevance to neuropsychiatric disorders. Brain Res Bull 2016;126:334–46.

[4] Johnson EB, Byrne LM, Gregory S, et al. Neurofilament light protein in blood predicts regional atrophy in Huntington disease [Internet]. Neurology 2018. 10.1212/WNL.0000000000005005. Available from: http://www.neurology.org/lookup/doi/10.1212/WNL.0000000000005005.

[5] Sjogren M, Blomberg M, Jonsson M, et al. Neurofilament protein in cerebrospinal fluid: a marker of white matter changes. J Neurosci Res 2001;66(3):510–6.

[6] Hampel H, Toschi N, Baldacci F, et al. Alzheimer's disease biomarker-guided diagnostic workflow using the added value of six combined cerebrospinal fluid

candidates: Aβ1–42, total-tau, phosphorylated-tau, NFL, neurogranin, and YKL-40. Alzheimer's Dement 2018;14(4):492–501.

[7] Rossi D, Volanti P, Brambilla L, et al. CSF neurofilament proteins as diagnostic and prognostic biomarkers for amyotrophic lateral sclerosis. J Neurol 2018;265 (3):510–21.

[8] Feneberg E, Oeckl P, Steinacker P, et al. Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. Neurology 2018;90(1):e22–30.

[9] Oeckl P, Jardel C, Salachas F, et al. Multicenter validation of CSF neurofilaments as diagnostic biomarkers for ALS [Internet]. Amyotroph Lateral Scler Front Degener 2016;17(5–6):404–13. Available from: https:// www.tandfonline.com/doi/full/10.3109/21678421.2016.1167913.

[10] Bergman J, Dring A, Zetterberg H, et al. Neurofilament light in CSF and serum is a sensitive marker for axonal white matter injury in MS [Internet]. Neurol – Neuroimmunol Neuroinflammation 2016;3(5):e271. Available from: http:// nn.neurology.org/lookup/doi/10.1212/NXI.0000000000000271.

[11] Goossens J, Bjerke M, Van Mossevelde S, et al. Diagnostic value of cerebrospinal fluid tau, neurofilament, and progranulin in definite frontotemporal lobar degeneration. Alzheimer's Res Ther 2018;10(1).

[12] Meeter LHH, Gendron TF, Sias AC, et al. Poly(GP), neurofilament and grey matter deficits in C9orf72 expansion carriers. Ann Clin Transl Neurol 2018;5 (5):583–97.

[13] Meeter LH, Dopper EG, Jiskoot LC, et al. Neurofilament light chain: a biomarker for genetic frontotemporal dementia [Internet]. Ann Clin Transl Neurol 2016;3 (8):623–36. Available from: http://doi.wiley.com/10.1002/acn3.325.

[14] Pijnenburg YAL, Verwey NA, van der Flier WM, et al. Discriminative and prognostic potential of cerebrospinal fluid phosphoTau/tau ratio and neurofilaments for frontotemporal dementia subtypes. Alzheimer's Dement Diagnosis Assess Dis Monit 2015;1(4):505–12.

[15] Van Eijk JJJ, Van Everbroeck B, Abdo WF, et al. CSF neurofilament proteins levels are elevated in sporadic Creutzfeldt-Jakob disease. J Alzheimer's Dis 2010;21(2):569–76.

[16] Zerr I, Schmitz M, Karch A, et al. Cerebrospinal fluid neurofilament light levels in neurodegenerative dementia: evaluation of diagnostic accuracy in the differential diagnosis of prion diseases [Internet]. Alzheimer's Dement 2018;9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29391125%0Ahttp:// linkinghub.elsevier.com/retrieve/pii/S1552526018300025.

[17] Zerr I, Kallenberg K, Summers DM, et al. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain 2009;132(Pt 10):2659–68.

[18] Parchi P, Giese A, Capellari S, et al. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999;46(2):224–33.

[19] Hermann P, Laux M, Glatzel M, et al. Validation and utilization of amended diagnostic criteria in Creutzfeldt-Jakob disease surveillance [Internet]. Neurology 2018. 10.1212/WNL.0000000000005860. Available from: http:// www.neurology.org/lookup/doi/10.1212/WNL.0000000000005860.

[20] Zerr I, Bodemer M, Gefeller O, et al. Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. Ann Neurol 1998;43(1):32–40.

[21] DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach [Internet]. Biometrics 1988;44(3):837. Available from: http://www. jstor.org/stable/2531595?origin=crossref.

[22] Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinf 2011;12.

[23] Llorens F, Schmitz M, Karch A, et al. Comparative analysis of cerebrospinal fluid biomarkers in the differential diagnosis of neurodegenerative dementia. Alzheimers Dement 2015:1–13.

[24] Schmitz M, Ebert E, Stoeck K, et al. Validation of 14-3-3 protein as a marker in sporadic Creutzfeldt-Jakob disease diagnostic. Mol Neurobiol 2015.

[25] Kovacs GG, Andreasson U, Liman V, et al. Plasma and cerebrospinal fluid tau and neurofilament concentrations in rapidly progressive neurological syndromes: a neuropathology-based cohort. Eur J Neurol 2017.

[26] Steinacker P, Blennow K, Halbgebauer S, et al. Neurofilaments in blood and CSF for diagnosis and prediction of onset in Creutzfeldt-Jakob disease [Internet]. Sci Rep 2016;6:38737. Available from: http://www.nature.com/articles/ srep38737.

[27] Abu-Rumeileh S, Capellari S, Stanzani-Maserati M, et al. The CSF neurofilament light signature in rapidly progressive neurodegenerative dementias. Alzheimer's Res Ther 2018;10(1).

[28] Cramm M, Schmitz M, Karch A, et al. Stability and reproducibility underscore utility of RT-QuIC for diagnosis of Creutzfeldt-Jakob disease. Mol Neurobiol 2016;53(3):1896–904.

[29] Schmitz M, Cramm M, Llorens F, et al. The real-time quaking-induced conversion assay for detection of human prion disease and study of other protein misfolding diseases [Internet]. Nat Protoc 2016;11(11):2233–42. Available from: http://www.nature.com/doifinder/10.1038/nprot.2016.120.

[30] McGuire LI, Peden AH, Orru CD, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease [Internet]. Ann Neurol 2012;72(2):278–85. Available from: http://www.ncbi.nlm. nih.gov/pubmed/22926858.

[31] Llorens F, Kruse N, Schmitz M, et al. Evaluation of α-synuclein as a novel cerebrospinal fluid biomarker in different forms of prion diseases. Alzheimer's Dement 2017;13(6):710–9.

[32] Ritter A, Cummings J. Fluid biomarkers in clinical trials of Alzheimer's disease therapeutics. Front Neurol 2015;6.

[33] Caverzasi E, Mandelli ML, DeArmond SJ, et al. White matter involvement in sporadic Creutzfeldt-Jakob disease. Brain 2014;137(12):3339–54.

[34] Grau-Rivera O, Calvo A, Bargalló N, et al. Quantitative magnetic resonance abnormalities in Creutzfeldt-Jakob disease and fatal insomnia. J Alzheimer's Dis 2016;55(1):431–43.