Diagnostic accuracy of CSF neurofilament light chain protein in the biomarker-guided classification system for Alzheimer's disease

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ABSTRACT

Objective. We assessed the diagnostic accuracy of the neurofilament light chain (NFL) protein in cerebrospinal fluid (CSF) in distinguishing patients with Alzheimer's disease (AD) from cognitively healthy controls (HCs) and frontotemporal dementia (FTD) patients. In particular, we tested the discriminatory performance of CSF NFL concentration in differentiating patient groups stratified by biomarker profiles, independent of the severity of cognitive impairment, using a novel unbiased biomarker-guided descriptive classification system for AD.

Methods. CSF NFL concentrations were examined in a multicenter cross-sectional study of 108 participants stratified in AD pathophysiology-negative (both amyloid beta and tau) (n = 15), tau-positive only (n = 15), amyloid beta-positive only (n = 13), AD pathophysiology-positive (n = 33), FTD (n = 9) patients, and HCs (n = 23), according to an innovative biomarker-based classification system.

Results. CSF NFL distinguished AD pathophysiology-positive patients from HCs, tau-positive only patients from HCs, and AD pathophysiology-positive patients from FTD with AUROCs = 0.77, 0.69, 0.54, respectively.

Conclusions. CSF NFL discriminated AD pathophysiology-positive patients from HCs with fair diagnostic accuracy, whereas the diagnostic accuracy in differentiating tau-positive patients from HCs is poor. Finally, the diagnostic accuracy in distinguishing AD pathophysiology-positive patients from FTD is unsatisfactory.

KEY WORDS: Neurofilament light chain protein; axonal degeneration; Alzheimer's disease; Alzheimer's disease pathophysiology; frontotemporal dementia; cognitive aging; mild cognitive impairment; biomarkers; biomarker-based diagnosis; cerebrospinal fluid.

INTRODUCTION

The canonical pathophysiological hallmarks of Alzheimer's disease (AD) are a) extracellular deposition of aggregated amyloid beta (Aβ - i.e. amyloid plaques), and b) intraneuronal accumulation of neurofibrillary tangles (NFTs)[1]. NFTs are primarily composed of hyperphosphorylated microtubule-binding protein tau (p-tau) as well as neurofilaments (NFs). NFs are neuron-specific heteropolymers[2], mostly expressed in large-caliber myelinated axons[3], and are key structural components of the neuronal cytoskeleton. One specific NF subunit, the neurofilament light chain (NFL) protein, is currently being examined as a candidate biomarker for the diagnosis of neurodegenerative diseases[4–7]. Accordingly, increased cerebrospinal fluid (CSF) concentrations of NFL (CSF NFL) have been detected in both clinical AD[4,5] and frontotemporal dementia (FTD)[6,7] compared to healthy controls.

The aim of this study was to assess the ability of CSF NFL to discriminate groups of cognitively impaired patients stratified across the AD pathophysiology *spectrum* via an unbiased classification system (i.e. independently of the degree of cognitive impairment)[8]. The "A/T/N" scheme employs three binary biomarker categories which reflect AD pathophysiology, where "A" refers to "amyloid-beta (Aβ) pathology", "T" to "tau pathology", and "N" to neurodegeneration. Specifically, we evaluated the diagnostic accuracy of NFL in distinguishing AD pathophysiology patients (i.e. patients showing decreased CSF concentrations of $A\beta_{1-42}$ and increased CSF concentrations of total tau (t-tau) or p-tau[9]) as well as patients with evidence of tau pathology only, from cognitively healthy controls (HCs). Furthermore, we evaluated the capability of CSF NFL to differentiate AD pathophysiology from FTD.

METHODS

Study participants

The research was designed as a multicenter cross-sectional study conducted retrospectively in a convenience series from three independent European academic expert memory clinic centers. A

total of 135 participants were examined; out of these individuals, 27 were excluded due to missing data in one or more CSF biomarkers. The remaining 108 were included in the present study: 35 participants were recruited from the Institute of Memory and Alzheimer's Disease (Institut de la Mémoire et de la Maladie d'Alzheimer, IM2A) at Pitié-Salpêtrière University Hospital in Paris (France), 57 from the German Center for Neurodegenerative Diseases (DZNE) in Rostock (Germany), and 16 from the Institute of Neuroscience and Physiology at Sahlgrenska University Hospital in Mölndal (Sweden). The study was conducted in accordance with the tenets of the Declaration of Helsinki and approved by the local Ethical Committees at each participating university center. All participants or their representatives gave written informed consent for the use of their clinical data for research purposes.

The STARD criteria for the reporting of diagnostic test accuracy studies (available at http://www.equator-network.org/reporting-guidelines/stard/) were followed.

Patient stratification and biomarker assessment

Patients received either a) a clinical diagnosis of AD dementia according to the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) consensus criteria[10], b) a clinical diagnosis of mild cognitive impairment (MCI) according to the MCI core clinical criteria of the National Institute on Aging-Alzheimer's Association (NIA-AA) guidelines[11], or c) a diagnosis of FTD following the consensus on clinical diagnostic criteria of 1998[12]. Cognitively HCs were individuals who volunteered for a lumbar puncture. Inclusion criteria were the absence of neurological/psychiatric disease history and a Mini-Mental State Examination (MMSE) score between 27 and 30. Our patient population was dissected according to the A/T/N scheme, an unbiased biomarker-based descriptive classification system recently proposed by Jack and colleagues[8]. The detailed description of patient stratification according to the A/T/N scheme, CSF sampling, immunoassays

for both CSF core biomarkers and CSF NFL protein, and statistical analysis are reported in the **Supplementary Materials**.

Statistical analysis

Age-, sex-, and site- adjusted NFL values were compared across groups through nonparametric statistics, after which areas under the ROC curves (AUROCs) for all binary classification problems were computed through logistic regression within a Leave-One-Out Cross-Validation (LOO-CV) approach. The discriminatory ability of NFL to correctly allocate participants to diagnostic groups was categorized as follows: "excellent" (AUROC 0.90–1.00), "good" (AUROC 0.80–0.89), "fair" (AUROC 0.70–0.79), "poor" (AUROC 0.60–0.69), or "fail"/no discriminatory capacity (AUROC 0.50–0.59)[13].

RESULTS

Table 1 summarizes group-wise concentrations of all analytes as well as demographic and clinical data. Cognitively HCs (Group 1) and AD pathophysiology-negative patients (Group 2, [A-T-N-]) were significantly younger than all other groups. Compared with HCs, CSF NFL concentrations were significantly higher in Group 3 [A-/T±/N+, A-/T+/N±] ($P = 0.014$) and Group 5 [A+/T±/N+, A+/T+/N±] ($P = 0.006$). Groups 3 and 5 presented significantly higher CSF NFL concentrations compared to Group 2, [A-/T-/N-] ($P = 0.015$ and $P = 0.006$, respectively) and Group 4, [A+/T-/N-] ($P = 0.015$ and $P = 0.0016$, respectively) (**Figure 1A**). CSF NFL differentiated cognitively HCs from Group 3 and Group 5 with AUROCs = 0.69 (95% CI, 0.50–0.87) (**Figure 1B**) and 0.77 (95% CI, 0.64–0.89) (**Figure 1C**), respectively. CSF NFL distinguished between Group 5 and Group 6 (FTD) with AUROC = 0.54 (95% CI, 0.28–0.80) (**Figure 1D**). Equivalent results obtained when stratifying according to purely clinical diagnostic criteria are reported in the **Supplementary Materials**.

DISCUSSION

Compared with HCs, we found significantly higher CSF NFL concentrations in all tau-positive patient categories. In turn, these patients presented significantly higher CSF NFL concentrations compared to AD pathophysiology-negative patients. Also, CSF NFL differentiated cognitively HCs from tau pathology-positive only patients with a barely fair/poor diagnostic accuracy and from AD pathophysiology-positive patients with a fair diagnostic accuracy.

These findings confirm the presence of a remarkable association between NFL and the neuronal injury marker tau[4,5]. Notably, NFL is primarily expressed in large-caliber myelinated axons[14], and is therefore assumed to be a marker of white matter disease. Increased CSF NFL concentrations are associated with white matter damage and other varieties of lesions in subcortical brain areas[15]. Moreover, a longitudinal study found that CSF NFL concentrations are higher in AD and MCI with respect to HCs, and that CSF NFL concentrations correlate with structural brain alterations and cognitive decline over time[5]. This indicates that CSF NFL is a progression marker in AD and MCI and suggests that large-caliber axonal disintegration is a prominent aspect of AD pathophysiology[5]. This was further substantiated in a recent comprehensive meta-analysis[4].

In addition, CSF NFL concentration observed in all tau-positive patient categories was higher than in Aβ pathology-positive patients. This result is supported by a pivotal study showing similar concentrations of CSF NFL in $A\beta_{1-42}$-negative and $A\beta_{1-42}$-positive individuals, as dichotomized by a cut-off value (CSF $A\beta_{1-42}$<192pg/mL). Consequently, alterations in CSF NFL concentrations do not seem to be dependent on the amyloidogenic $A\beta_{1-42}$ peptide[5] since they are substantially correlated with biomarkers of neurodegeneration, such as brain atrophy, and cognitive decline[16].

In contrast to a previous study[7], the ability of CSF NFL to discriminate between AD pathophysiology-positive patients and FTD was unsatisfactory (**Figure 1D**) . Additional analyses across a range of different neurodegenerative diseases are needed to shed more light on this aspect.

The study has some potential caveats that need to be reported. Because of the relatively small sample size, it was not possible to stratify the cohort into all groups established by the original A/T/N scheme[8]. Therefore, given that only validated core CSF biomarkers (i.e. no neuroimaging) were employed, MCI and AD dementia individuals were grouped into a single category (**Supplementary Figure 1**). Because of the cross-sectional nature of the study, it was not possible to differentiate potentially stable-MCI cases from those converting into dementia as well as to report on prognosis and rate of progression of cognitive impairment. Moreover, extensive psychometric data were not available, thus preventing the study of CSF NFL concentrations in relation to different cognitive measures. Additionally, the quantification of the core CSF biomarkers of AD was not performed in a centralized manner and, while we controlled for center effects in our statistical analysis, further inter-laboratory variability cannot be entirely ruled out.

This study is largely exploratory and is a first attempt to employ NFL protein as a CSF biological marker for AD diagnosis by employing an original, unbiased biomarker-based system of classification[8]. Conceptually, the A/T/N dissection model addresses the need for a unifying approach to using biomarkers in the study of AD. Due to its unbiased nature, the A/T/N scheme represents a flexible model that could be employed in any framework of existing diagnostic criteria[8]. Potentially, it is expected to integrate key and novel evolving biomarkers of other relevant pathophysiological mechanisms belonging to the *spectrum* of age-related neurodegenerative diseases, as well as genetic or epigenetic factors.

In conclusion, our multicenter cross-sectional study highlights the diagnostic accuracy of CSF protein NFL in differentiating AD pathophysiology-positive patients from HCs using an innovative unbiased classification system which allows a biomarker-driven stratification of patients and is independent of the severity of cognitive impairment.

Additional studies are required to examine whether CSF NFL may be employed as a biological indicator of mechanism of action and/or target engagement or as a marker predicting progression of cognitive impairment in drug development studies.

CONTRIBUTORS

SL: study concept and design, analysis and interpretation of data, drafting of the manuscript; NT: study concept and design, statistical analysis, analysis and interpretation of data, critical revision of the manuscript for important intellectual content; FB: study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content; HZ: acquisition of data, critical revision of the manuscript for important intellectual content; KB: acquisition of data, critical revision of the manuscript for important intellectual content; IK: acquisition of data, critical revision of the manuscript for important intellectual content; SJT: acquisition of data, critical revision of the manuscript for important intellectual content; EC: analysis and interpretation of data, critical revision of the manuscript for important intellectual content; AMS: acquisition of data, critical revision of the manuscript for important intellectual content; SE: acquisition of data, critical revision of the manuscript for important intellectual content; FL: acquisition of data, critical revision of the manuscript for important intellectual content; BD: acquisition of data, critical revision of the manuscript for important intellectual content; RF: critical revision of the manuscript for important intellectual content; FG: critical revision of the manuscript for important intellectual content; HH: study concept and design, study supervision, analysis and interpretation of data, critical revision of the manuscript for important intellectual content.

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COMPETING INTERESTS

SL received lecture honoraria from Roche. HZ and KB are co-founders of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg. SE received lecture honoraria from Roche and participated on scientific advisory boards of GE Healthcare and Eli Lilly. BD reports personal fees from Eli Lilly. HH reports no conflict of interest with the content of the present manuscript. He serves as Senior Associate Editor for the Journal *Alzheimer's & Dementia*; he has been a scientific consultant and/or speaker and/or attended scientific advisory boards of Axovant, Anavex, Eli Lilly and company, GE Healthcare, Cytox Ltd, Jung Diagnostics GmbH, Roche, Biogen Idec, Takeda-Zinfandel, Oryzon Genomics; and he receives research support from the Association for Alzheimer Research (Paris), Pierre and Marie Curie University (Paris), Pfizer & Avid (paid to institution); and he has patent applications, but receives no royalties. NT, FB, IK, SJT, EC, AMS, FL, RF, FG declare no conflicts of interest.

ETHICS APPROVAL

The study was conducted in accordance with the tenets of the Declaration of Helsinki and approved by the local Ethical Committees at each participating university center. All participants or

their representatives gave written informed consent for the use of their clinical data for research purposes.

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