Evaluation of CSF neurofilament light chain levels as a routine biomarker in a memory clinic.

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Abstract

Systematic evaluation of biomarkers in representative populations is needed to validate their clinical utility. In this work, we assessed the diagnostic performance of cerebrospinal fluid neurofilament light chain (NfL) in a neurocognitive clinical setting. A total of 51 patients with different cognitive clinical syndromes and 11 cognitively normal individuals were evaluated in a memory clinic in Argentina. Clinical conditions included: Mild cognitive impairment (MCI, n=12), Dementia of Alzheimer's Type (DAT, n=14), Behavioral variant Frontotemporal dementia (bvFTD, n=13), and Primary Progressive Aphasia [logopenic (lvPPA, n=6), semantic (svPPA, n=2), and non-fluent (nfvPPA, n=4)]. We quantified CSF NfL and core Alzheimer's disease biomarkers using commercially available ELISA kits. Cortical thickness was analyzed on brain MRI scans from 10 controls and 10 patients. CSF NfL was significantly increased in MCI, FTD and DAT patients compared to controls (Kruskal-Wallis, $p < 0.0001$). Interestingly, receiver operating characteristic (ROC) curve analysis showed the highest area under the curve (AUC) value when analyzing control versus bvFTD patients (AUC= 0.9441). Also, we observed a marginally significant correlation between NfL levels and left orbito-frontal cortex thickness in a small group of patients with FTD. Overall, our results further support CSF NfL as a promising biomarker in the diagnostic work up of bvFTD.

Keywords: biology of aging, dementia, neurodegeneration, Latin America

INTRODUCTION

Diagnosis of Alzheimer's disease (AD) and frontotemporal dementia (FTD) in the clinic relies on careful neurological examination, neuropsychological testing, laboratory studies to rule out reversible causes of cognitive impairment and, oftentimes, structural brain MRI. In 2011, the NIA-AA put forward research recommendations for revising diagnostic criteria for AD¹. These new criteria include analysis of core AD biomarkers ($A\beta$, total tau, and phospho-tau₁₈₁) given their pivotal role in improving diagnostic accuracy².

Core AD biomarkers have been used in the differential diagnosis of atypical AD from bvFTD³ and in distinguishing underlying AD or FTD pathology in cases of primary progressive aphasia (PPA)⁴. Apart from these molecules, few fluid biomarkers have proven useful and specific for FTD diagnosis and monitoring. One promising biomarker for FTD is neurofilament light chain (NfL). Neurofilaments are neuronal-specific intermediate filaments composed of 4 subunits, which play important roles in structural support, transport and neurotransmission⁵. NfL is abundantly expressed in subcortical brain regions, specifically in large-caliber myelinated axons⁶. Therefore, increased NfL is regarded as a marker of white matter alterations and several subcortical lesions^{7,8}. Also, a number of studies analyzing either autopsy-confirmed or genetic cases have shown cerebrospinal fluid (CSF) NfL to be significantly increased in FTD compared to healthy controls^{9,10}. However, NfL's ability to discriminate between FTD and AD has been shown in some studies^{10,11}, but not in others¹². Of note, among FTD cases higher NfL levels have been associated with disease severity^{13,14} and, specifically in genetic cases, progranulin mutation carriers exhibited higher NfL compared to *C9orf72* expansion carriers⁹. Taken together, these

evidences strongly support NfL as a useful biomarker in the diagnostic work up of cognitive syndromes in the clinic.

However, in order for NfL to transition from research to routine clinical practice, additional evidence of its performance in different populations is sorely needed. Here, we present the first Latin American experience in incorporating cerebrospinal fluid NfL analysis to the existing panel of core AD biomarkers in a specialized memory clinic.

METHODS

Subjects

Participants enrolled in this study (total: 62) were evaluated at the Memory and Aging Center (FLENI, Buenos Aires, Argentina). Control individuals (n=11) were part of the Argentina-ADNI cohort. Individuals in this group did not exhibit cognitive impairment at the time of examination showing Mini Mental Status Examination (MMSE) scores ranging from 24 to 30 and a Clinical Dementia Rating score of 0. Participation in the ADNI protocol involved neuropsychological examinations, CSF biomarker analysis, DNA extraction and neuroimaging studies. On the other hand, the group of patients with clinical cognitive syndromes were either referred by their primary care physician or made a first consultation at our center because of cognitive complaints. Clinical diagnoses were made according to standard criteria and included: Dementia of Alzheimer's Type¹⁵ (DAT, n=14), Behavioral variant Frontotemporal Dementia¹⁶ (bvFTD, n=13), Mild Cognitive Impairment¹⁷ (MCI, n=12), and Primary progressive aphasia¹⁸ (PPA) including: logopenic (lvPPA, n=6), semantic (svPPA, n=2), and non-fluent (nfvPPA, n=4) variants. All cases in this study were sporadic. After evaluation, patients were invited to participate in a research

protocol to validate CSF and imaging biomarkers. Written informed consent was obtained from all subjects and/or legal representatives. This study was approved by the institutional Ethics Committee.

Cerebrospinal fluid (CSF) analysis

CSF was obtained by lumbar puncture (LP), in the morning, using a 22G Sprotte needle. LP was performed at level L3/L4 or L4/L5 and CSF (at least 4 ml) was collected in polypropylene tubes. Samples were centrifuged at 2000xg during 10 minutes. Supernatants were stored in polypropylene cryovials at -80°C until analysis.

Amyloid-beta 1-42 ($A\beta_{1-42}$), total-tau (t-tau), and phospho-tau (p-tau) were quantified using INNOTEST immunoassays from Fujirebio (Belgium)¹⁹, following instructions from the manufacturer.

NfL quantification was performed using the NF-Light Assay from Uman Diagnostics (Sweden) according to manufacturer's instructions²⁰.

Magnetic Resonance Imaging and Cortical Thickness calculation

MRI was performed on a 3.0T scanner (Signa HDxt 3.0T, GE Healthcare, Milwaukee, WI) with an 8-channel head coil. 3D-SPGR T1-WI cortical reconstruction and volumetric segmentation was performed with FreeSurfer image analysis suite v6.0 and cortical thickness was analyzed between patients and controls in different anatomical regions of interest. For the Alzheimer's disease (AD) group, brain regions included: hippocampus, parietal, and frontal lobes. For the FTD group, we analyzed the following regions: superior frontal, orbito frontal, caudal middle frontal, rostral middle frontal, inferior and superior

parietal lobes. Mean cortical thickness and standard deviation for each region of interest was calculated for the 10 control individuals ($\bar{x}_C$ and SD_C , respectively). For each patient (6 in the AD group and 4 in the FTD group) we calculated differences in cortical thickness relative to control according to the formula: $(\bar{x}_p + 2SD_C) - \bar{x}_C$, where $\bar{x}_p$ is the mean cortical thickness in the patient. We assumed *a priori* that cortical thicknesses in patients were lower than in controls.

Statistical analyses:

Kruskal-Wallis test was used for comparisons among different clinical categories. Mann-Whitney test was used for comparisons between two clinical categories. To determine NfL diagnostic accuracy we used receiver operating characteristic (ROC) curve analysis. Spearman correlation coefficient was calculated using the R Software. All other tests were performed with GraphPad Prism 5.

RESULTS AND DISCUSSION

Here we present the first report on CSF NfL analysis in a group of Latin American patients with different cognitive clinical syndromes. Demographic and clinical characteristics of participants are summarized in Table 1. As expected, patients with bvFTD exhibited younger age at lumbar puncture (LP) than MCI or DAT patients while there were no statistically significant differences in ages between MCI, DAT and control groups. Duration of cognitive complaint until LP did not differ significantly between groups.

Core AD biomarker analysis showed decreased $A\beta_{1-42}$ in MCI and DAT compared to controls (Table 1), consistent with the notion that patients in these categories are in the

Alzheimer's pathophysiology continuum²¹. Notably, total-tau was only increased in DAT patients while phospho-tau (p-tau) levels showed no significant differences in either MCI or DAT compared to controls. Thus, according to the recently proposed A/T/(N) classification scheme and the NIA-AA research framework²¹, DAT patients in our cohort would correspond to the category "Alzheimer's and concomitant suspected non-Alzheimer's pathologic change with dementia".

CSF NfL levels were significantly increased in all clinical categories compared to controls (Table 1 and Figure 1). This is in agreement with previous reports showing that NfL is a reliable marker of neurodegeneration^{10,11}. In this regard, we observed that NfL discriminated MCI patients from controls whereas total-tau did not. Further studies would be needed to assess the usefulness of either or both biomarkers of neuronal injury/neurodegeneration in this clinical category. Moreover, NfL levels were significantly higher in bvFTD compared to DAT or MCI patients (Figure 1), in agreement with other studies^{11,13,14}. Similar to observations in these reports, we found a wide variance in NfL levels within the bvFTD category which could be due to different underlying pathologies. In one series, NfL levels were higher in tau-negative compared to tau-positive cases¹¹. NfL levels differed among PPA subtypes but the low sample number precluded any statistical analysis. To determine NfL's performance in discriminating clinical categories, we measured the area under receiver operating characteristic (ROC) curves. The highest area under the curve (AUC) was reached when analyzing Control versus bvFTD (AUC=0.944, $p<0.001$, 95% CI 0.85-1.04). The optimal cutoff was 1157 pg/ml (84.62% sensitivity, 90.91% specificity). This result supports a role for NfL in differentiating bvFTD from non-neurodegenerative conditions with behavioral involvement, (e.g., psychiatric disorders).

This is in line with a previous report²² that showed NfL to have a good diagnostic accuracy in distinguishing bvFTD from primary psychiatric disorders (AUC=0.93, $p<0.001$).

Also, NfL exhibited an excellent discriminatory capacity in control versus DAT cases (AUC=0.922 $p<0.001$, 95% CI 0.82-1.03), similar to other reports¹²⁻¹⁴. On the other hand, NfL only fairly discriminated between bvFTD and DAT (AUC=0.736, $p=0.037$, 95% CI 0.54-0.94). Goossens *et al*⁹ also found NfL to be higher in bvFTD compared to AD in a larger cohort while, Lista *et al*¹² did not find significant differences between these two categories. Overall, these results warrant further studies in larger well-defined cohorts in order to establish a definitive role for NfL in differentiating bvFTD from AD.

Magnetic resonance imaging (MRI) scans were available from 10 control individuals, 6 patients with a CSF biomarker profile compatible with Alzheimer's disease (AD group), and 4 patients grouped as FTD (3 bvFTD and one semantic variant primary progressive aphasia, FTD group). To evaluate a possible correlation between CSF NfL levels and atrophy in relevant brain regions, we measured cortical thickness in control individuals and patients from the AD and FTD groups. Interestingly, we only observed a marginally significant negative correlation between NfL levels and left orbito-frontal cortical thickness in the FTD group (Spearman $r = -1$, $p=0.083$). Although not statistically significant, this biologically relevant observation adds to the existing evidence that increased NfL levels correlate with increased left frontal lobe atrophy in patients with svPPA and nvPPA but not with lvPPA²³. Also, studies on genetic frontotemporal dementia showed NfL to be negatively correlated with whole brain volume and with frontal, temporal, parietal, insular and cingulate cortices²⁴.

Although preliminary, our results offer additional evidence on the usefulness of CSF NfL in the diagnostic discrimination between sporadic bvFTD and psychiatric conditions. Our work has several caveats such as the low sample number, lack of confirmatory neuropathology, and the fact that it is a cross-sectional study. Thus, the role of NfL in predicting rate of progression or severity cannot be determined in this series. Also, the fact that NfL levels differ widely among FTD patients could reflect distinct underlying proteinopathies. Interestingly, it has been recently shown that tau can physiologically enter neurons²⁵, suggesting a novel role for a cytoskeleton protein in intercellular communication. It is tempting to speculate that NfL could exhibit a similar function. Thus, pathological changes associated with specific subtypes of FTD could differentially disrupt NfL flow between cells, and ultimately its steady-state levels, regardless of neuronal loss.

Also, NfL together with core AD biomarker analysis could aid in the differential diagnosis of atypical variants of AD and FTD. Finally, multicenter NfL analysis in different populations are needed to contribute to the widespread standardization and implementation of this biomarker in the diagnostic work up of FTD.

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Table 1. Demographic and clinical characteristics of participants

	Control	MCI	bvFTD	DAT	<i>P</i> -value ^a	nfvPPA	svPPA	lvPPA
n	11	12	13	14		4	2	6
Sex, F/M	6/5	5/7	9/4	7/7		3/1	0/2	4/2
Age at lumbar puncture, years, median, (range)	63 (53-75)	69.5 (62-77)	58 (39-70)	71 (53-81)	0.002 ^b 0.077 ^c	60 (51-68)	61 (56-66)	62 (57-66)
Disease duration at time of LP, years, median, (range)	-	9.5 (1-13)	3.0 (0.6-20)	6.5 (0.3-11)	0.418 ^d	7.5 (3-12)	2.38 (0.75-4)	3.0 (1-7)
CSF A β ₁₋₄₂ , pg/ml mean (SD)	777.6 (282.7)	569.9 (315.6)	866.6 (236.7)	414.3 (123.5)	0.002 ^e <0.001 ^f	660.9 (410.1)	489.9 (264.2)	274.4 (95.0)
CSF t-tau, pg/ml mean (SD)	217.3 (109.6)	242.6 (185.7)	235.2 (132.4)	380.3 (202.0)	0.029 ^g	565.0 (448.1)	283.2 (154.5)	296.3 (180.0)
CSF p-tau, pg/ml mean (SD)	38.2 (16.1)	44.2 (34.0)	38.0 (14.91)	46.9 (15.5)	0.348 ^h	67.0 (58.3)	50.2 (32.1)	43.9 (12.7)
CSF NfL, pg/ml, mean (SD)	656.6 (286.9)	1176.9 (598.5)	2609.5 (1684.9)	1335.0 (432.9)	<0.001 ⁱ	3173.5 (2157.6)	1400.1 (655.9)	943.2 (199.5)

Note. Abbreviations: “MCI”: Mild cognitive impairment, “bvFTD”: Behavioral variant frontotemporal dementia, “DAT”: Dementia of Alzheimer’s Type, “nfvPPA”: Non-fluent variant primary progressive aphasia, “svPPA”: Semantic variant primary progressive aphasia, “lvPPA”: Logopenic variant primary progressive aphasia, “LP”: Lumbar puncture “CSF”: Cerebrospinal fluid, “t-tau”: total tau, “p-tau”: phospho-tau T181, “NfL”: Neurofilament light chain, “SD”: Standard deviation

^a Due to the low sample number, primary progressive aphasia were not included in the statistical analyses

^b p -value for all clinical categories (Kruskal-Wallis)

^c p -value excluding FTD (Kruskal Wallis)

^d p -value using Kruskal-Wallis

^e p -value for Control versus MCI (Mann Whitney)

^f p -value for Control versus DAT (Mann Whitney)

^g p -value for Control versus DAT (Mann Whitney)

^h p -value for all clinical categories (Kruskal-Wallis)

ⁱ p -value for all clinical categories (Kruskal-Wallis)

LEGEND TO FIGURE 1

Figure 1. Quantification of cerebrospinal fluid (CSF) Neurofilament light chain (NfL) in different cognitive clinical syndromes. NfL was increased in each clinical category compared to controls. bvFTD showed significant increased NfL compared to DAT and MCI. No significant differences were observed between MCI and DAT. Non-parametric Mann-Whitney test was performed for paired clinical categories and the exact p value is shown for each comparison. Abbreviations: “MCI”: Mild cognitive impairment, “bvFTD”: Behavioral variant frontotemporal dementia, “DAT”: Dementia of Alzheimer’s Type, “nfvPPA”: Non-fluent variant primary progressive aphasia, “svPPA”: Semantic variant primary progressive aphasia, “lvPPA”: Logopenic variant primary progressive aphasia

Figure 1