Plasma neurofilament light chain in memory clinic practice: Evidence from a real-life study

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ABSTRACT

Objective: To explore the accuracy of plasma neurofilament light chain (NfL) as a biomarker for diagnosis and staging of cognitive impairment, in a large cohort with of previously diagnosed patients in clinical practice.

Methods: Retrospective, cross-sectional, monocentric study, from a tertiary memory clinic. Patients underwent cerebrospinal fluid core Alzheimer’s disease (AD) biomarker evaluation using ELISA or Elecsys methods, and plasma NfL analysis using the single molecule array technology. The patients’ biomarker data were examined for associations with: i/cognitive status ii/presence of neurodegenerative disease and iii/diagnostic groups. Associations between core CSF biomarkers and plasma NfL were determined.

Results: Participants (N = 558, mean age = 69.2 ± 8.8, 56.5% women) were diagnosed with AD (n = 274, considering dementia and MCI stages), frontotemporal dementia (FTD, n = 55), Lewy body disease (LBD, n = 40, considering MCI and dementia stages), other neurodegenerative diseases, n = 57 (e.g Supranuclear Palsy, Corticobasal syndrome), non-neurodegenerative cognitive impairment (NND, n = 79, e.g. vascular lesions, epilepsy or psychiatric disorders) or subjective cognitive impairment (SCI, n = 53). Mean plasma NfL (log, pg/mL) levels were higher in neurodegenerative than non-neurodegenerative disorders (1.35 ± 0.2 vs 1.16 ± 0.23, p < 0.001), and associated with the stage of cognitive impairment (p < 0.001). The addition of plasma NfL to a clinical model (age, MMSE and APOE ε4 carriership) marginally improved the discrimination of degenerative from non-degenerative disorders in ROC analysis (AUC clinical model: 0.81, 95% CI = [0.77;0.85], AUC clinical model + plasma NfL: AUC = 0.83 95% CI = [0.78;0.87], delta Akaike information criterion = −11.7).

Discussion: Plasma NfL could help discrimination between degenerative and non-degenerative cognitive disorders, albeit not better than comprehensive clinical evaluation.

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Abbreviations: AD, Alzheimer’s disease; ALS, amyotrophic lateral sclerosis; CBS, corticobasal syndrome; CJD, Creutzfeldt-Jakob disease; CSF, cerebrospinal fluid; LBD, Lewy body disease; FTD, fronto-temporal dementia; HIV, Human immunodeficiency virus; HD, Huntington disease; LATE, limbic-predominant age-related TDP-43 encephalopathy; MCI, mild cognitive impairment; MS, multiple sclerosis; MSA, multiple system atrophy; MRI, magnetic resonance imaging; pNfL, plasma neurofilament light chain; NND, non-neurodegenerative disease; OND, other neurodegenerative disease; PD, Parkinson’s disease; PDD, Parkinson’s disease Dementia; PSP, progressive supranuclear palsy; p-Tau, phosphorylated tau protein; SCI, Subjective cognitive impairment.

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1. Introduction

Neurofilaments light chain (NFL) are cytoskeletal components of neuronal axons, mainly present in large caliber myelinated axons, known to play a determinant role in the axon radial growth and stability (Khall et al., 2018). Within the last decade, new assay methods, such as single molecule array (Simoa), allowed ultra-sensitive blood measurements of this cerebral biomarker, unreachable with prior ELISA techniques (Rühle et al., 2016).

Plasma NFL was shown to reflect axonal damage in selected study cohorts of neurodegenerative disorders: Alzheimer’s disease (AD) (Mattsson et al., 2017), frontotemporal dementia (FTD) (Rohrer et al., 2016; Ashton et al., 2021; Benussi et al., 2020), or Lewy body disease (LBD) (Ashton et al., 2021; Pilotto et al., 2021), with good accuracy for the discrimination of neurodegenerative diseases from controls (Pilotto et al., 2021). We summarized the published body of evidence regarding NFL in this scope in Supplementary Table 1.

However, in daily clinical practice, patients often report unspecific cognitive complaints or clinical symptoms, leading to two unmet needs: i/ to easily differentiate neurodegenerative from non-neurodegenerative disorders (NND), ii/ to help the clinicians refine early preliminary diagnoses, among neurodegenerative conditions. So far, little is known about the discriminating power of plasma NFL in daily clinical practice, in unselected populations with all causes of cognitive impairment, without excluding older patients, or those in advanced stages of dementia.

Our objectives were firstly to compare plasma NFL levels across clinical diagnoses, with regards to the presence or absence of neurodegeneration, in a sample of previously diagnosed patients from daily clinical practice and secondly to study plasma NFL levels, across different stages of cognitive impairment.

2. Methods

2.1. Study design and participants

This retrospective, cross-sectional study, included patients with cognitive complaints who had undergone cerebrospinal fluid (CSF) analysis at the Cognitive Neurology Center, Lariboisière (GHU AP-HP Nord, Paris), between 01/2010 and 02/2021. This department has expertise in diagnosis and care of patients with cognitive disorders and neurodegenerative diseases.

2.1.1. Diagnosis assessment

Patients were assessed by a multidisciplinary team of dementia experts, along three lines: i/ the cognitive status (impaired or not); ii/ the presence of underlying neurodegenerative process; iii/ the final etiological diagnosis. Accordingly, diagnostic groups were established after consideration of the clinical presentation, neuropsychological assessment, neuroimaging and CSF biomarkers, using the most recent diagnostic criteria (Gorno-Tempini et al., 2011; Crutch et al., 2017; McKhann et al., 2011; McKeith et al., 2020; McKeith et al., 2017; Hermann et al., 2021; Armstrong et al., 2013; Höglinger et al., 2017; Rascovsky et al., 2011) (See Supplementary Table 2). All patients diagnosed with AD were on the AD continuum (Mild Cognitive Impairment (MCI) and dementia) and had a biomarker profile with abnormal Aβ40/Aβ42 ratio (Jack et al., 2018).

We enrolled 558 participants: individuals with subjective cognitive impairment (SCI, n = 53), with no evidence of neurocognitive disorder, AD (n = 274), FTD (n = 55), LBD (n = 40), other neurodegenerative disease (OND, n = 57) and patients with non-neurodegenerative disease (NND, n = 79). Regarding cognitive status, 53 were SCI subjects, 218 individuals had MCI and 287 had dementia. Overall, 426 individuals (76.4%) had a neurodegenerative disease in the whole cohort.

In details, the OND group included patients with a diagnosis of dementia related to Creutzfeldt Jakob disease (Hermann et al., 2021), supranuclear palsy (Höglinger et al., 2017), corticobasal syndrome (Armstrong et al., 2013), logopenic variant primary progressive aphasia (Gorno-Tempini et al., 2011), as well as patients with dementia with evidence of neurodegeneration (defined by atrophy on morphological brain MRI, or hypometabolism on Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) imaging, or suspected non amyloid pathologic profile according to CSF biomarkers with cognitive decline over follow-up).

The NND group included subjects who i/ experienced objective cognitive decline; ii/ did not fulfill clinical criteria for the main etiologies of neurodegenerative dementia (see Supplementary Table 2); iii/ did not evoke another cause of uncommon neurodegenerative condition, after multidisciplinary consultation including a neuroradiologist, a nuclear physician and careful reviewing of neuroimaging examinations (e.g. FDG-PET imaging, MRI), as well as CSF biomarkers. Therefore, the final diagnoses of the NND group comprised vascular cognitive impairment, sleep apnoea, alcohol-related cognitive impairment, epilepsy, psychiatric disorder (bipolar disorder, depression), cognitive impairment linked to prior traumatic brain injury, infectious diseases (human immunodeficiency viruses, herpes viruses), metabolic (B12 vitamin deficiency, thyroid dysfunction) and toxic (e.g. chemotherapy treatment) cognitive impairment.

SCI individuals reported cognitive complaints without evidence for cognitive impairment or neurodegeneration, and might have been included as control subjects, in previous observational research studies. They were classified as SCI when a diagnostic of neurocognitive disorder was excluded by the referent physician and they fulfilled the following criteria: i/ the neuropsychological assessment found preserved global cognition (i.e. normative or subnormative scores for age, sex and level of education; ii/ brain MRI did not show significant hippocampal atrophy (Scheltens score ≤ 2); iii/ the CSF biomarker profile was normal (A-T-N-), according to the AT(N) classification (Rascovsky et al., 2011).

For clarity purpose, we stated as “degenerative conditions” AD, FTD, LBD, OND and as “non-degenerative conditions” NND and SCI in the analyses described below.

2.2. Non-inclusion criteria

Patients were not included when no consensual etiological diagnosis was reached or when patients had undergone plasma NFL analysis, after an acute neurological event (traumatic brain injury, stroke).

The flow chart of study participants is presented on Fig. 1.

2.3. Plasma NFL and CSF biomarker measurements

Plasma NFL levels were measured in singlicates, using the Simoa platform (Quanterix®, Lexington, MA) in Lariboisière Hospital, and in Neurochemistry Laboratory (Moldaln, Sweden), across 10 analytical runs. Each assay plate included internal quality control samples with high and low plasma NFL concentrations, which were analyzed in duplicate at the beginning and end of the plate. Intra-assay and inter-assay coefficients of variation (CV) were respectively of 3.1% and 11.8%. CV between the two platforms was computed from a subset of 10 samples run on both, and rendered a CV of 7.5%.

CSF Aβ ratio (Aβ42/Aβ40), phosphorylated-tau (pTau) and total tau (Tau) measurements were performed in the Biochemistry Unit (Lariboisière Hospital), using Innotest® ELISA (FujiRebio, Gent, Belgium) (2010–2018), and after 2018 using Elecsys® immunoassays on the cobas e601 analyzer (Roche-Diagnostics). (See cut off levels in Supplementary Table 3).

2.4. Statistical analyses

Patients’ data was analyzed along three lines: i/ the cognitive status (SCI, MCI and dementia) ii/ according to the presence of neurodegenerative disease or not and iii/ across diagnoses group: AD, LBD, FTD,
OND, NND and SCI. The accuracy of plasma NfL, in addition with clinical assessment, to discriminate neurodegenerative from non-neurodegenerative conditions was assessed with receiver operating characteristic curves (ROC) and multiple logistic regression models. Age, MMSE and APOE ε4 carriership were associated in a clinical model; then a total model included plasma NfL and the aforementioned variables. Akaike information criterion (AIC) was used to determine which model was the best fit for the data. A difference in AIC > 2 points between two models (ΔAIC) was considered as significant to indicate better fit of the model with lower AIC.

Plasma NfL concentration was log-transformed to achieve normality, except in the logistic regression analysis. Differences in plasma NfL across multiple category variables were tested using analysis of variance (ANOVA), followed by post-hoc test with Bonferroni correction for multiple analyses. CSF pTau and Tau were expressed as a ratio relative to the standard used at the time of the assay for the calculation of the correlation with plasma NfL, in order to avoid false associations due to change of cut off values for CSF AD biomarkers. (See supplementary Table 1 for used cut of values). Correlation between continuous variables, such as pNfL and core CSF biomarkers were analyzed using Spearman or Pearson correlation coefficients. Categorical variables were compared using χ² test. \( P < 0.05 \) was considered overall significant. Statistical analyses were performed using IBM SPSS 27.0 and GraphPad Prism 9.1.3.

2.5. Ethical considerations

All the participants were provided oral and written information about the opportunity to collect additional blood and CSF samples for further research analyses, in the BioCogBank© protocol. They also consented for the anonymous use of their clinical data and the results of their CSF analyses). This study was approved by the local and national Ethics Committees (“Comité d’évaluation et d’Ethique pour la recherche Paris Nord” on 30 May 2016) and the “Commission Nationale Informatique et Libertés” (CNIL).

3. Results

3.1. Characteristics of the study sample

The patients’ characteristics and plasma NfL values are displayed in Table 1. The relationship between plasma NfL and the cognitive status, the degenerative or non-degenerative status and the diagnostic groups is illustrated on Fig. 2. Plasma NfL was correlated with age (rho = 0.40, \( P < 0.0001 \)) but not with sex (\( P = 0.66 \)) nor with APOE ε4 carriership (\( P = 0.36 \)).

3.2. Plasma NfL regarding cognitive status

Plasma NfL was associated with the severity of cognitive impairment, regardless of the etiology (Table 1). Plasma NfL levels were higher in subjects with MCI or dementia vs SCI, \( P < 0.001 \). There was also a stepwise increase between patients with MCI and dementia (\( P < 0.001 \)), as shown on Fig. 2A.

3.3. Plasma NfL regarding degenerative or non-degenerative status

Plasma NfL levels in neurodegenerative patients were higher than in non-neurodegenerative patients, as reported on Fig. 2B (mean ± sd 1.16 ± 0.23 versus 1.35 ± 0.32 pg/mL, \( P < 0.001 \)). Mean age was significantly higher in patients with neurodegenerative disorders (mean ± sd 70.8 ± 8.0) than in those with non-neurodegenerative conditions (mean ± sd 64.8 ± 9.6), \( P < 0.0001 \). In ROC analysis, plasma NfL discriminated neurodegenerative from non-neurodegenerative conditions with an AUC of 0.75, 95% CI = [0.69;0.80], \( P < 0.0001 \). Our clinical model (age, sex and MMSE) yielded an AUC of 0.81, 95% CI = [0.77;0.85] to identify neurodegenerative conditions. The association of plasma NfL to the clinical variables in the total model significantly improved the diagnostic accuracy: AUC = 0.83 95% CI = [0.78;0.87] (AIC versus clinical model: -11.7) (Table 2 and Fig. 3).

3.4. Plasma NfL regarding diagnostic groups

Plasma NfL levels were higher in all diagnostic groups compared with SCI. Although displaying the higher levels in our study, plasma NfL...
levels from individuals with FTD did not differ statistically from AD or OND. However, plasma NfL was higher in FTD than in NND or LBD. LBD patients’ plasma NfL levels were lower than individuals with AD (p = 0.0014), FTD (p < 0.001) but did not differ from subjects with NND (Fig. 2C). The levels of plasma NfL between patients with MCI vs dementia in FTD were significantly different (1.28 ± 0.21 vs. 1.48 ± 0.24, respectively p < 0.001 after age-adjustment), whereas the difference was not significant in MCI vs. dementia due to AD.

After adjustment on age, sex and MMSE levels, plasma NfL was significantly higher in patients with AD, FTD and OND than in SCI individuals (p = 0.0002, p = 0.0004, p = 0.0016 respectively). Patients with LBD and NND did not differ significantly from SCI individuals. Patients with AD and FTD had higher plasma NfL levels than patients with NND, without outpassing multiple comparison correction. The detailed between-group comparisons of NfL levels are presented in Supplementary Table 4.

3.5. Relation between Plasma NfL and core CSF biomarkers

Plasma NfL and AD CSF biomarkers were significantly correlated, with a Pearson correlation coefficient of 0.27 between plasma NfL and t-tau (p < 0.001), 0.22 with p-Tau (p < 0.001), and 0.17 with Aβ40/Aβ42 ratio.

4. Discussion

This analysis of 558 patients, from daily clinical practice, highlighted the potential interest of plasma NfL as a reliable biomarker, along the diagnostic process. First, plasma NfL accurately reflected the stage of cognitive impairment, regardless of the diagnosis; plasma levels were higher in subjects with dementia than in those with MCI, and higher in MCI patients than in SCI. Second, plasma NfL was higher in patients with neurodegenerative disorders than in those with non-neurodegenerative conditions. The addition of plasma NfL to clinical variables (age, MMSE and APOE ε4 status) marginally improved the discrimination of neurodegenerative and non-neurodegenerative conditions. Third, patients with FTD showed the highest plasma NfL concentration, which differed significantly from individuals diagnosed with LBD, SCI, and NND, but not from those with OND or AD. In a recent publication, Wilke et al. reported that plasma NfL progressively increased in conversion stages of genetic forms of FTD (Wilke et al., 2022). The absence of difference between patients with FTD and AD might be explained by the inclusion of early-stage FTD subjects in our sample (Davy et al., 2021). We confirmed the results of prior publications (Ashton et al., 2021; Pilotto et al., 2021) showing that patients with LBD presented higher levels of plasma NfL than SCI, but also lower levels than patients with AD, FTD or OND.

To the best of our knowledge, only two other studies assessed the role of plasma NfL in cognitively impaired patients with different diagnostic groups. Last year, Ashton et al. established optimal cutpoints for plasma NfL in 2269 individuals with various neurodegenerative or NND, achieving the correct classification of most individuals with FTD, progressive supranuclear palsy, multiple system atrophy, or amyotrophic lateral sclerosis (Ashton et al., 2021). However, in this study, participants came from two multicenter cohorts (KCL in the UK and BioFINDER in Sweden). Because of stringent inclusion and exclusion criteria, such as thresholds of MMSE for MCI and dementia staging, the population of these studies did not truly reflect the daily clinical practice settings and concerns. Willemse et al. highlighted the interest of plasma NfL, as a biomarker of neurodegenerative diseases, in a smaller sample (n = 109) of younger patients (mean age 63 ± 9) from clinical practice, especially in the youngest half of their study population (Willemse et al., 2021). In our population, plasma NfL considered alone, was less accurate to rule out a psychiatric condition (Davy et al., 2021). Yet, assessing
plasma NfL in case of diagnostic doubt between AD and OND would be useless for the clinician.

Plasma NfL levels modifications in neurodegenerative disorders are related to chronic and progressive neuronal death (Bacioglu et al., 2016). Thus, unlike in brain injuries (stroke, meningitis), plasma NfL levels progressively increase over the course of the disease, reflecting the dynamic process of neurodegeneration. This dynamic profile of plasma NfL was confirmed in clinical studies assessing the therapeutic response to AD and FTD treatment (Bacioglu et al., 2016).
Table 2

<table>
<thead>
<tr>
<th>Logistic regression model</th>
<th>AUC</th>
<th>Odds ratio</th>
<th>95% Confidence interval limits</th>
<th>Δ AIC versus Clinical model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Model (Age, Apo E4 carriership, MMSE)</td>
<td>0.81</td>
<td>0.77</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.09</td>
<td>1.06</td>
<td>1.12</td>
<td></td>
</tr>
<tr>
<td>APOE E4 carriership</td>
<td>4.01</td>
<td>2.41</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>MMSE</td>
<td>0.86</td>
<td>0.81</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Plasma NfL</td>
<td>0.75</td>
<td>0.69</td>
<td>0.80</td>
<td>66.6</td>
</tr>
<tr>
<td>Plasma NfL</td>
<td>1.078</td>
<td>1.05</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>Total model (plasma NfL, Age, Apo E4 carriership, MMSE)</td>
<td>0.83</td>
<td>0.78</td>
<td>0.87</td>
<td>−11.70</td>
</tr>
</tbody>
</table>

Logistic regression was performed to study the performance of clinical variables (age, MMSE and APOE E4 carriership), plasma NfL and of their association for identifying patient with a neurodegenerative conditions. Comparisons between AUCs were performed using Aikake information criterion (AIC). A difference in AIC (Δ AIC) of >2 is considered to indicate a significant difference between the models.

AIC, Aikake information criterion; ApoE, apolipoprotein E; AUC, area under the curve; MMSE, mini mental state examination; NfL: neurofilament light chain.

and disease activity in patients with multiple sclerosis (Kühle et al., 2019). Our results also suggest the potential use of this dynamic biomarker in clinical practice, for the monitoring of unselected patients, in the field of neurocognitive disorders, as patients are frequently referred by neurologists to tertiary memory centers with the perspective of participation to clinical trials. Similarly, the number of patients with non-neurodegenerative conditions was smaller than the number of patients with degenerative conditions, thus oversizing the weight of high plasma NfL values for this group. Patients with non-neurodegenerative disorders were significantly younger than those with neurodegenerative conditions. Yet, our analyses were adjusted for age. Moreover, this was a cross-sectional analysis with only one plasma NfL measurement by participant. Thus, there are uncertainties regarding the evolution of plasma NfL in our participants over the years of follow-up.

5. Conclusion

In this clinical practice study, plasma NfL was significantly higher in neurodegenerative conditions and associated with the severity of cognitive impairment. However, NfL marginally improved discrimination between neurodegenerative and non-neurodegenerative disorders when added to clinical evaluation. Thus, plasma NfL could help physicians experiencing diagnostic uncertainty, in patients with atypical clinical presentation, to confirm or exclude neurodegeneration. Yet, NfL may not be regarded as a routine diagnostic biomarker of neurodegeneration, to be implemented in memory clinics daily practice. Further studies with more patients affected by NND are warranted to provide external validity to our findings. The relationship of plasma NfL to other progression biomarkers, such as neuroimaging abnormalities, also deserves further investigation.

Ethical authorizations and consent

All the participants were provided oral and written information about the opportunity to collect additional blood and CSF samples for further research analyses, in the BioCogBank© protocol. They also consented for the anonymous use of their clinical data and the results of their CSF analyses. The “Comité d’Evaluation de l’Ethique des projets de Recherche Biomédicale (CEERB) Paris Nord” (Institutional Review Board-IRB 00006477–of HUPNVS, Paris 7 University, AP-HP), has reviewed and approved the research project entitled «Clinico-biological database of cognitive disorders» (Dr Dumurgier, principal investigator) on May 30, 2016, and the “Commission Nationale Informatique et Libertés” (CNIL).

Data availability statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.
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None

Authors contribution
KG, ML and CP designed the study and processed the data of participants. KG, AV and ML drafted the manuscript. KG and AV performed the NfL measurements; MM, EBA, and FML supervised the procedure. JH, JD, EC, FML, EBA, KB, HZ, CP and ML critically reviewed and edited the manuscript and contributed to the discussion.

CRedit authorship contribution statement


Declaration of Competing Interest
KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. The other authors report no conflict of interest.

Data availability
Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2022.105937.

References