Association of plasma neurofilament light chain with neocortical amyloid-β load and cognitive performance in cognitively normal elderly participants

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Abbreviations: Alzheimer's disease, AD; apolipoprotein E gene, *APOE;* cerebrospinal fluid, CSF; memory assessment clinic – questionnaire, MAC-Q; mild cognitive impairment, MCI; mini mental state examination, MMSE; Montreal cognitive assessment, MoCA; neocortical amyloid-β load, NAL; neurofilament light chain, NFL; positron emission tomography, PET; standard uptake value ratio, SUVR; subjective memory complainers, SMC

Abstract (250/(250 words max))

Background: Neurofilament light chain (NFL), an axonal cytoskeletal protein reported to maintain neuronal integrity, is elevated in the cerebrospinal fluid and blood in Alzheimer's disease (AD).

Objective: Investigate the association of plasma NFL with preclinical-AD features, such as high neocortical amyloid-β load (NAL) and subjective memory complaints, and cognitive performance in cognitively normal older adults.

Methods: Plasma NFL concentrations were measured employing the single molecule array (Simoa) platform in participants from the KARVIAH cohort, aged 65-90 years. Participants underwent a battery of neuropsychological testing to evaluate cognitive performance and were categorised as low NAL (NAL-, n=65) and high NAL (NAL+, n=35) assessed via positron emission tomography, and further stratified into subjective memory complainers (SMC; nNAL-=51, nNAL+=25) and non-SMC (nNAL-=14, nNAL+=10) based on the Memory Assessment Clinic–Questionnaire (MAC-Q).

Results: Plasma NFL inversely correlated with global cognition, verbal and visual episodic memory and, executive function and working memory. No significant difference in NFL was observed between NAL+ and NAL- participants, however within *APOEε4* non-carriers, higher NAL was observed in individuals with NFL concentrations within quartiles3 and 4(vs

quartile1). Additionally, within the NAL+ participants, SMC had a trend of higher NFL compared to non-SMC.

Conclusion: Plasma NFL is inversely associated with cognitive performance in elderly individuals. While plasma NFL may not reflect NAL in individuals with normal global cognition, the current observations indicate that it may be used to track onset of axonal injury in NAL+ individuals. It could thus potentially serve as a biomarker to tell when amyloid pathology has entered the neurotoxic phase.

Introduction

Neurofilament (NF) is a crucial axonal cytoskeletal component comprising three subunits, namely, neurofilament light chain (NFL), neurofilament medium chain (NFM) and neurofilament heavy chain (NFH) [1]. The disruption of NF in neuronal damage occurring within neurodegenerative conditions, results in the release of NF into the cerebrospinal fluid (CSF) [2]; consequently giving rise to elevated NFL concentrations in the CSF, as has been reported in Alzheimer's disease (AD) [3] and other neurodegenerative diseases [4-7].

With the invasive nature of CSF collection via lumbar puncture, the potential of employing NFL as a biomarker for disease diagnosis or progression, and possibly for screening, further drove the investigation of blood NFL alterations, in disease [2, 8-10]. Employing the Alzheimer's Disease Neurodegenerative Initiative (ADNI) cohort, Mattsson and colleagues reported significantly higher plasma NFL in AD and mild cognitively impaired (MCI) patients compared to controls [11]. Additionally, higher plasma NFL has been associated with compromised cognition and hippocampal atrophy both cross-sectionally and longitudinally. Furthermore, plasma NFL was inversely associated with brain glucose metabolism, longitudinally in the same highly characterized ADNI cohort [11]. More recently, a study conducted on autosomal dominant Alzheimer's disease (ADAD) families found that plasma NFL was higher in the asymptomatic mutation carriers (aMC) and symptomatic mutation carriers (sMC) compared to their non-carrier (NC) relatives, wherein plasma NFL

concentrations were reported to be about 1.3-fold higher in aMC and 3.6-fold higher in sMC [12].

However, plasma NFL in individuals with preclinical AD prior to cognitive impairment, characterised by neocortical amyloid-β load (NAL) measured via positron emission tomography (PET) and subjective memory complaints, has not been investigated previously. Furthermore, the association between plasma NFL and cognitive performance in cognitively normal elderly individuals has also not been examined.

Therefore, the current study investigated the association between plasma NFL and NAL, employing a standard uptake value ratio (SUVR) cut-off value of 1.35, categorising study participants as high NAL (SUVR≥1.35; NAL+) and low NAL (SUVR<1.35; NAL-), given that the aberrant build-up of NAL begins as early as two decades prior to the clinical manifestation of AD [13]. Based on the Memory Assessment Clinic–Questionnaire (MAC-Q) [14], NAL+ participants were further categorised into subjective memory complainers (SMC) and non-complainers (non-SMC) to examine NFL alterations within the preclinical cohort subset at highest risk of AD (NAL+ SMC). The current study also examined whether plasma NFL levels inversely correlated with cognitive performance within cognitively normal elderly individuals. Our investigations were therefore aimed at gaining better insight into whether axonal injury, reflected by increased plasma NFL, is present in the early pathogenesis of AD.

Methods

Participants

Study participants belonged to the Kerr Anglican Retirement Village Initiative in Ageing Health (KARVIAH) cohort, at baseline. All participants were residents of Anglicare, New South Wales, Australia.

Cohort volunteers (N=206) were required to meet the set screening inclusion and exclusion criteria to be eligible for the KARVIAH cohort. Briefly, the inclusion criteria for the KARVIAH cohort comprised an age range of 65-90 years, good general health, no known significant cerebral vascular disease, fluent in English, adequate/corrected vision and hearing to enable testing, no objective cognitive impairment as screened by a Montreal Cognitive Assessment (MoCA) score ≥26. MoCA scores lying between 18-25 were assessed on a case by case basis by the study neuropsychologist following stratification of scores according to age and education [15]. The exclusion criteria comprised, the diagnosis of dementia based on the revised criteria from the National Institute on Aging - Alzheimer's Association [16], presence of acute functional psychiatric disorder (including lifetime history of schizophrenia or bipolar disorder), history of stroke, severe or extremely severe depression (based on the depression, anxiety, stress scales; DASS) and uncontrolled hypertension (systolic BP > 170 or diastolic BP > 100).

One hundred and five participants out of the 134 volunteers meeting the inclusion/exclusion criteria, underwent neuroimaging, neuropsychometric evaluation and blood collection, as the remaining either declined undergoing neuroimaging or withdrew from the study. Within these 105 participants, 100 participants were considered to have normal global cognition based on their Mini-Mental State Examination score [17] (MMSE ≥26), and were included in the current study. Plasma NFL concentrations were reported in all 100 participants considered to have normal global cognition. All volunteers provided written informed consent prior to participation, and the Bellberry Human Research Ethics Committee, Australia, provided approval for the study.

Evaluation of neocortical amyloid-β load via PET

All study participants were imaged within three months of blood collection. Participants underwent positron emission tomography (PET) using ligand ^{18}F-Florbetaben (FBB) at Macquarie Medical Imaging in Sydney. Participants were administered an intravenous bolus of FBB slowly over 30s, while in a rested position. Images were acquired over a 20 min scan, in 5 min acquisitions, beginning 50 min post injection. Neocortical amyloid-β load was calculated as the mean SUVR of the frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions using image processing software, CapAIBL [18, 19].

Blood collection, APOE genotyping, measurement of plasma NFL

All study participants fasted for a minimum of 10 hours overnight prior to blood withdraw employing standard serological methods and processing [20]. Apolipoprotein E (*APOE)* genotype was determined from purified genomic DNA extracted from 0.5 ml whole blood as previously described [20].

Plasma NFL concentrations were measured employing the ultra-sensitive single-molecule array (Simoa) platform [10, 11]. Calibrators were run in duplicates and samples were run in singlicates with a 4-fold dilution. Two quality control (QC) levels were run in duplicates at the beginning and the end of each plate. For QC with concentration 12.1 pg/mL, repeatability and intermediate precision were 20.2 % while for QC with concentration 155.8 pg/mL, repeatability was 14.6 % and intermediate precision was 14.9 %. The lowest limit of quantification was 6 pg/mL.

Neuropsychological tests

All study participants underwent a comprehensive battery of neuropsychological testing. The full battery comprised the MoCA [15], MMSE [17], MAC-Q [14], Rey Auditory Verbal Learning Test (RAVLT) [21], Logical Memory (LM) I and II (WMS-III; Story A only) [22],

Rey Complex Figure Test (RCFT)[23], Wechsler Adult Intelligence Scale – Third edition (WAIS–III) Digit Span [24], WAIS–III Digit Symbol Substitution Test (DSST) [25], D-KEFS Category Fluency (Boys Names) and Switching (Fruits and Furniture) Tasks [26], Controlled Oral Word Association Test [27], Stroop Test (Victoria version) [28], the Boston Naming Test [29], Wechsler Test of Adult Reading [30] and the DASS [31]. Composite scores were generated for verbal and visual episodic memory and for working memory and executive function. The verbal and visual episodic memory composite score was created from the mean of the Z-scores of RAVLT List A, RAVLT short delay, RAVLT long delay, LM I, LMII, RCFT 3 min and RCFT 30 min while the working memory and executive function composite score was generated from the mean of the Z-scores of Digit Span backward, DSST, D-KEFS Boys names and Fruits and Furniture Switching tasks. The global composite score was constructed from the mean of the Z-score measures of RAVLT List A, RAVLT short delay, RAVLT long delay, LM I, LM II, RCFT 3 min, RCFT 30 min, Digit Span backward, DSST, D-KEFS Boys names and Fruits and Furniture Switching tasks and MMSE.

Statistical analyses

Descriptive statistics including means and standard deviations were calculated for NAL+ and NAL- groups. Chi-square tests were employed to compare the frequency of gender, *APOE ε4* carrier status and SMC between NAL+ and NAL- groups. Additionally, linear models were employed to compare continuous variables examined in the study between groups of interest (e.g. NAL- vs. NAL+, NAL+/non-SMC vs. NAL+/ SMC, NFL quartiles Q1 vs. Q2, Q3, Q4 etc.) with and without adjusting for covariates age, gender and *APOE ε4* carrier status. Continuous response variables were tested for approximate normality and variance homogeneity, and log transformed when required to satisfy test criteria. Pearson's correlation coefficient was employed to investigate correlations between NFL and other continuous

variables of interest, except for the correlation between NFL and MMSE where Spearman's correlation was used. Partial correlations were used when associations investigated were adjusted for age. All analyses were carried out using IBM® SPSS® Version 23.

Results

Demographic characteristics of study participants have been represented in Table 1, wherein no significant differences were observed between NAL- and NAL+ participants, except for the expected observation of a significantly higher *APOE* ε4 carrier frequency in the NAL+ group.

Plasma NFL was observed to correlate with age (r=.533, p<.0001) while no significant association between plasma NFL was observed with education (r= -.004, p=.965), gender (mean±SD: males, 39.26±20.55 pg/mL; females, 35.02±16.30 pg/mL; p=.268) or *APOE* ε4 carriage (mean±SD: non-carriers, 37.49±18.29 pg/mL; carriers, 32.18±15.39 pg/mL; p=.226).

Plasma NFL was not observed to be significantly elevated in NAL+ participants (or preclinical AD) compared to NAL- participants (mean±SD: NAL-, 35.23±17.27 pg/mL; NAL+, 38.50±18.75 pg/mL) with (p=.563) and without (p=.384) adjusting for age, gender and *APOE* ε4, however, a trend of elevated plasma NFL concentrations was observed in NAL+ SMC compared to NAL+ non-SMC, adjusting for age, gender and *APOE* ε4 carrier status (mean±SD, NAL+/non-SMC (n=10): 31.50±12.57 pg/mL, NAL+/SMC (n=25): 41.30±20.26 pg/mL; p=.069).

Additionally, after stratifying the cohort into *APOE* ε4 carriers (n=21) and non-carriers (n=79), within the *APOE* ε4 non-carrier subset, participants carrying NFL concentrations lying within quartile 1 were observed to have significantly lower NAL (1.15±0.13) compared to those in quartiles 3 (1.32±0.29, p=.031) and 4 (1.38±0.32, p=.006, Figure 1). However, after adjusting for age, significance disappeared between quartile 1 and 3, and only a trend remained between

quartile 1 and 4 (p=.068), likely due to the strong correlation between NFL and age, confounding associations of NFL with other age-related outcomes within the scope of this modest sample size.

Plasma NFL correlated inversely with verbal and visual episodic memory (r= -.305, p=.002) and working memory and executive function (r= -.357, p=.0002) in all participants (Figure 2). Plasma NFL was also observed to correlate inversely with global cognition assessed via MMSE (r= -.326, p=.001) and the global composite score (r= -.407, p<.0001) (Figure 2). On adjusting for age, plasma NFL continued to significantly correlate inversely with working memory and executive function (r= -.200, p=.047) and the global composite score (r= -.223, p=.026).

Discussion

Plasma NFL concentrations were not significantly elevated in cognitively normal elderly NAL+ versus NAL- participants. Our findings are in line with those reported by Mattsson and colleagues wherein no significant difference in plasma NFL was observed between NAL- (based on CSF Aβ42 ≥192 ng/L, n=71) and NAL+ (based on CSF Aβ42 <192 ng/L, n=41) cognitively normal participants [11]. Furthermore, associations between brain atrophy and plasma (and CSF) NFL concentrations reported previously in MCI, AD (and cognitively normal) individuals with both high and low CSF Aβ load [32], indicate that NFL is a general neurodegeneration biomarker, with high levels in many disorders such as PSP and FTD (Ref – se intro), which also is consistent with the observations of NFL between NAL+ and NAL- participants in the current study. However, we observed a trend of elevated plasma NFL in SMC compared to non-memory complainers within the NAL+ subset employed in the current study - the cohort subset likely to be the most advanced in the preclinical AD pathogenesis trajectory.

Interestingly, Mattsson and colleagues, also observed that MCI and AD *APOE* ε4 non-carriers had significantly higher plasma NFL concentrations compared to ε4 carriers. Within the current study as well, *APOE* ε4 non-carriers had a higher mean plasma NFL (37.49 pg/ml) compared to *APOE* ε4 carriers (32.18 pg/ml), although it did not reach significance presumably due to the relatively small numbers in the latter group. However, within the *APOE* ε4 non-carrier cohort subset, a trend of higher NAL was observed in participants with plasma NFL lying within Q3 and Q4 compared to Q1, suggesting that the onset of axonal cytoskeletal disruption may commence as early as this preclinical phase of AD.

Within the current study we did not observe a significant correlation between plasma NFL and hippocampal atrophy (data not shown): an observation in line with findings reported by Pereira and colleagues, wherein plasma NFL was associated with brain atrophy only in symptomatic cases, while CSF NFL concentrations were associated with brain atrophy in AD, MCI and cognitively normal subjects [32]. Similar observations have also been reported in the autosomal dominant form of AD (ADAD) by Weston and colleagues [12]. No association between plasma NFL and neocortical glucose metabolism (data not shown) was observed within the current cross-sectional study, which is in agreement with the findings reported by Mattsson and colleagues [11].

Plasma NFL was observed to correlate strongly with age in the present study. This correlation is consistent with previous studies reporting significant correlations between age and NFL in both, CSF and plasma (or serum) from healthy controls, individuals with pre-symptomatic neurodegenerative disease and within cohorts comprising healthy controls, MCI and AD patients [3, 7, 9, 11, 33, 34].

Additionally, plasma and CSF NFL concentrations have previously been reported to be inversely associated with MMSE, the AD assessment scale–cognitive subscale, Clinical

Dementia Rating Scale and the Trail Making Test-B scores in participants with MCI, sporadic AD, ADAD and bipolar disorder [3, 11, 12, 35]. The current study also observed that plasma NFL correlated inversely with cognitive performance, particularly, verbal and visual episodic memory, executive function and working memory and global cognition suggesting that higher plasma NFL concentrations are associated with inferior cognitive performance in elderly individuals with normal global cognition as well.

We acknowledge that the current study has limitations with regard to its relatively modest sample size. However, the present study also has its strengths given that it utilizes a highly characterised, cognitively normal cohort with a representative proportion of preclinical AD individuals, in agreement with other established cohorts [36, 37], employing PET for NAL measurement, a stronger marker of AD neuropathology compared to CSF Aβ, as employed previously [11]. Additionally, the study also incorporates a comprehensive battery of neuropsychological tests and most importantly, a highly sensitive assay to measure plasma NFL. Further investigation of the association between NAL and NFL in cognitively normal elderly individuals via an orthogonal method, for example mass-spectrometry (MS), may be employed for validation given the differences between immune-assay based techniques and MS: Immune-based methods detect both modified and unmodified members of a specific protein species, as well as possible oligomeric forms.   but also potentially bind to other off-target proteins in the sample, as the monoclonal antibody recognises an epitope which may also be presented in an unrelated protein. In contrast, the exact primary sequence of the peptide being measured is known in MS, however this technology only measures those peptides that are unmodified therefore restricting itself to a subset of all the isoforms of a specific protein carrying that sequence. Additionally, differences between native folded protein and denatured protein also contribute to disparities in measurements of the same protein employing orthogonal techniques.

To summarise, plasma NFL was not significantly higher in NAL+ versus NAL- cognitively normal elderly individuals, however a trend of elevated plasma NFL was observed within the NAL+ SMC (the cohort subset likely to be the farthest in the preclinical AD pathogenesis trajectory), indicating onset of axonal injury occurs well before the onset of clinical AD symptoms. Additionally, significant associations were observed between plasma NFL and neuropsychometric parameters representing visual and verbal memory, executive function and working memory and global cognition, in the current study. Our current plasma NFL observations along with those previously published indicate that plasma NFL alterations begin to manifest within the end stage of preclinical AD, and while NFL is not a sufficient stand-alone marker for preclinical and clinical AD, it has the potential to serve as an early marker of neurodegeneration. Moreover, given that plasma NFL correlated with cognition, it is a promising biomarker for disease progression and for monitoring disease modifying therapies, reaffirming the potential of elevated plasma NFL as a marker of progressive neurodegeneration.

Acknowledgements

Conflict of interest

KG and RNM are co-founders of the KaRa Institute of Neurological Diseases. RNM is the Founder of Alzhyme and owns stock in Alzhyme. HRS has received personal compensation for previous activities with Pfizer and currently with Takeda Pharmaceuticals. KB has served as a consultant or at advisory boards for Alzheon, BioArctic, Biogen, Eli Lilly, Fujirebio Europe, IBL International, Merck, Novartis, Pfizer, and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg. HZ has served at advisory boards of Roche Diagnostics and Eli Lilly, has received travel support from Teva and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company at the University of Gothenburg.

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