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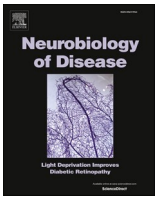

Cerebrospinal fluid neurofilament light chain is a marker of aging and white matter damage

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ABSTRACT

Background: Cerebrospinal fluid (CSF) neurofilament light chain (NfL) reflects neuro-axonal damage and is increasingly used to evaluate disease progression across neurological conditions including Alzheimer disease (AD). However, it is unknown how NfL relates to specific types of brain tissue. We sought to determine whether CSF NfL is more strongly associated with total gray matter, white matter, or white matter hyperintensity (WMH) volume, and to quantify the relative importance of brain tissue volume, age, and AD marker status (i.e., *APOE* genotype, brain amyloidosis, tauopathy, and cognitive status) in predicting CSF NfL.

Methods: 419 participants (Clinical Dementia Rating [CDR] Scale > 0, $N = 71$) had CSF, magnetic resonance imaging (MRI), and neuropsychological data. A subset had amyloid positron emission tomography (PET) and tau PET. Pearson correlation analysis was used to determine the association between CSF NfL and age. Multiple regression was used to determine which brain volume (i.e., gray, white, or WMH volume) most strongly associated with CSF NfL. Stepwise regression and dominance analyses were used to determine the individual contributions and relative importance of brain volume, age, and AD marker status in predicting CSF NfL.

Results: CSF NfL increased with age ($r = 0.59$, $p < 0.001$). Elevated CSF NfL was associated with greater total WMH volume ($p < 0.001$), but not gray or white matter volume (p's > 0.05) when considered simultaneously. Age and WMH volume were consistently more important (i.e., have greater R^2 values) than AD markers when predicting CSF NfL.

Conclusions: CSF NfL is a non-specific marker of aging and white matter integrity with limited sensitivity to specific markers of AD. CSF NfL likely reflects processes associated with cerebrovascular disease.

1. Introduction

Neurofilament light chain (NfL) is considered a biomarker of neuro-axonal injury and neurodegeneration and is increasingly used to evaluate disease progression across multiple neurological conditions, including Alzheimer disease (AD) (Yuan et al., 2017; Gaetani et al., 2019; Skillbäck et al., 2014; Gaiottino et al., 2013; Petzold et al., 2007; Gordon, 2020; Khalil et al., 2018). NfL is one of the scaffolding proteins of the neuronal cytoskeleton and plays a role in axonal and dendritic branching and growth. When axonal damage occurs, NfL levels increase in cerebrospinal fluid (CSF) and blood (Petzold, 2005). In addition to AD (Mattsson et al., 2017; Zetterberg et al., 2016; Preische et al., 2019; Zhou et al., 2017; Jin et al., 2019; Olsson et al., 2016), elevated NfL levels have been reported in multiple neurodegenerative disorders including stroke (Korley et al., 2019; Duering et al., 2018), Parkinson disease dementia (Bäckström et al., 2015), multiple sclerosis (Kuhle et al., 2019; Eikelenboom et al., 2003; Bergman et al., 2016), frontotemporal dementia (Landqvist Waldö et al., 2013), and amyotrophic lateral sclerosis (Lu et al., 2015).

Despite growing attention as a non-specific biomarker of neuro-axonal injury and neurodegeneration, little is known regarding whether CSF NfL preferentially relates to gray matter, white matter, and/or white matter hyperintensities [WMH]) in sporadic AD, and how brain amyloidosis and tauopathy influence these relationships. Previous research has demonstrated that elevated CSF NfL is independently associated with cortical thinning (Mattsson et al., 2017; Preische et al., 2019; Pereira et al., 2017) and faster accumulation of WMHs (Zetterberg et al., 2016) in individuals with mild cognitive impairment.

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Table 1
Participant characteristics.

	CDR 0	CDR > 0	*p*	Total
N	348	71		419
Age mean (SD), years	67.83 (8.4)	72.72 (5.9)	<0.001	68.65 (8.3)
Sex, *n* (% female)	203 (58.3%)	34 (47.9%)	0.11	56.6%
Race, *n* (% Non-Hispanic White)	317 (91.1%)	67 (94.4%)	0.50	91.6%
APOE ε4, *n* (% positive)	124 (35.6%)	39 (54.9%)	<0.01	38.9%
Amyloid PET status, *n* (%)				
Amyloid PET-	**223** (79.9%)	**12** (54.5%)	<0.001	**235** (78.1%)
Age mean (SD), years	65.81 (8.2)	67.56 (5.8)	0.47	65.89 (8.1)
Sex, *n* (% female)	137 (61.4%)	8 (66.7%)	0.72	145 (61.7%)
Race, *n* (% Non-Hispanic White)	202 (90.6%)	11 (91.7%)	1.00	213 (90.6%)
APOE ε4, % positive	63 (28.3%)	2 (16.7%)	0.38	65 (27.7%)
Amyloid PET+	**56** (20.1%)	**10** (45.5%)	<0.001	**66** (21.9%)
Age mean (SD), years	71.71 (7.0)	74.33 (5.5)	0.27	72.11 (6.8)
Sex, *n* (% female)	26 (46.4%)	3 (30.0%)	0.34	29 (43.9%)
Race, *n* (% Non-Hispanic White)	50 (89.3%)	10 (100.0%)	0.63	60 (90.9%)
APOE ε4, % positive	33 (58.9%)	7 (70.0%)	0.52	40 (66.7%)
Tau PET, *n (%)*				
Tau PET-	**45** (69.2%)	**4** (57.1%)	<0.001	**49** (68.1%)
Age mean (SD), years	67.03 (8.3)	71.03 (5.4)	0.42	67.28 (8.1)
Sex, *n* (% female)	20 (44.4%)	2 (66.7%)	0.47	23 (46.9%)
Race, *n* (% Non-Hispanic White)	43 (95.6%)	3 (100.0%)	1.00	47 (95.9%)
APOE ε4, % positive	11 (24.4%)	0 (0.0%)	0.34	11 (22.4%)
Tau PET+	**20** (30.8%)	**3** (42.9%)	<0.001	**23** (31.9%)
Age mean (SD), years	70.26 (6.6)	73.33 (5.0)	0.45	70.67 (6.4)
Sex, *n* (% female)	9 (45.0%)	2 (66.7%)	0.51	11 (47.8%)
Race, *n* (% Non-Hispanic White)	18 (90.0%)	3 (100.0%)	1.00	21 (91.3%)
APOE ε4, % positive	7 (35.0%)	1 (33.3%)	0.96	8 (34.8%)

Abbreviations: APOE ε4 = apolipoprotein E; CDR = Clinical Dementia Rating Scale; PET = positron emission tomography; MRI = magnetic resonance imaging. Amyloid positivity was defined as having a Centiloid value of 16.4 or greater. Tau positivity was defined as having an AV1451 value of 1.22 standardized uptake value ratio (SUVR) or greater. The mean length of time between the lumbar puncture (LP) and cognitive testing dates was 72.18 days (*SD* = 75.88); the mean length between LP and the MRI scan date was 7.69 days (*SD* = 156.44); the mean length between the LP and PET dates was 20.93 days (*SD* = 180.74); the mean length between MRI and cognitive testing was 79.87 (*SD* = 178.18); the mean length between MRI and PET was 13.24 days (SD = 131.37); the mean length between PET and cognitive testing was 93.11 days (SD = 201.27). Of the individuals who were APOE ε4+, 16 were ε2/ε4.

Furthermore, CSF NfL increases with age and elevated levels of CSF NfL are associated with increases in amyloid and tau in individuals with AD (Mattsson et al., 2017; Zetterberg et al., 2016; Jin et al., 2019). However, few studies consider the individual contributions of age and AD marker status (e.g., amyloid PET, tau PET, *APOE* genotype, and cognitive status) when examining associations with CSF NfL (Mattsson et al., 2017; Zetterberg et al., 2016; Zhou et al., 2017; Mattsson et al., 2016).

Using a sample of mostly cognitively normal individuals with or without preclinical AD, and very mild AD older adults, the main objectives of this study were to: a) assess the relationship between CSF NfL and age; b) quantify associations between CSF NfL and AD marker status c) determine whether CSF NfL more strongly associates with total gray matter, white matter, or WMH; and d) quantify the relative importance of brain volume, age, and AD marker status in predicting CSF NfL.

2. Methods and materials

2.1. Participants

Data were obtained from 419 individuals, aged 43–91, enrolled in memory and aging studies at the Knight Alzheimer Disease Research Center (ADRC) at Washington University in St. Louis, MO. Biomarker procedures and neuroimaging were performed at study entry and repeated every 2–3 years. From this sample, 76.6% were in their 60's and 70's (*n* 40's = 7; *n* 50's = 42; *n* 60's = 163; *n* 70's = 158; *n* 80's = 48; *n* 90's = 1). 384 participants were Non-Hispanic White and 35 were Black. Inclusion criteria for this study were that individuals had neuroimaging (PET, MRI) and CSF data collected within 3 years of the clinical and neuropsychological visit. The mean length of time between measures was 80 days (standard deviation = 178 days). Cognitive status and age were computed from the clinical and neuropsychological assessment date. A subset completed amyloid and/or tau positron emission tomography (PET) imaging. All procedures were approved by the Washington University Institutional Review Board, and each participant provided written informed consent.

2.1.1. Neuropsychological and clinical assessment

Individuals aged 65 years or older underwent clinical assessments annually while individuals aged 43 to 64 years old were assessed every 3 years. At each neuropsychological and clinical assessment, participants completed the Mini-Mental State Examination (MMSE) and were assigned a Clinical Dementia Rating (CDR) score by an experienced clinician. The CDR Sum of Boxes (CDR-SB), which is the combination of scores in sub-domains, was also recorded. A CDR of 0 indicates that the individual is cognitively normal, while CDR 0.5 and CDR 1 indicate very mild and mild AD, respectively (Morris, 1993). Participants were subsequently grouped as either CDR 0 or CDR > 0. All participants with a CDR > 0 had a clinical diagnosis of dementia of the Alzheimer's type in accordance with the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer Disease and Related Disorders Association (Buckner et al., 2009; McKhann et al., 1984; Sperling et al., 2009). Individuals with a dementia etiology other than AD were excluded from the analyses.

2.1.2. APOE ε4 status

DNA samples were collected at enrollment and genotyped using either an Illumina 610 or Omniexpress chip. *APOE* ε2, ε3, and ε4 isoforms were determined by genotyping rs7412 and rs429358 using established methods (Cruchaga et al., 2013). Participants were classified as either *APOE* ε4 positive (ε4/ε4, ε4/ε3, ε4/ε2) or *APOE* ε4 negative (ε2/ε2, ε2/ε3, ε3/ε3).

2.1.3. Cerebrospinal fluid analysis

After overnight fasting a lumbar puncture (LP) as performed in the morning by a trained neurologist. CSF (10–20 mL) was collected by gravity grip using a 22-gauge Sprotte spinal needle (Geisingen, Germany). CSF was aliquoted (500 ul) into polypropylene tubes. All samples were free of visible blood contamination. After collection, samples were centrifuged briefly (2000 *g* for 15 mins) to remove any cellular debris, transferred to another polypropylene tube, aliquotted and frozen at −80 °C until analysis. CSF was analyzed for NfL by enzyme-linked immunosorbent assay (Fagan et al., 2006; Henson et al., 2020).

2.1.4. Magnetic resonance imaging

Imaging was performed using either a 3.0 Tesla Siemens Biograph mMR (Erlangen, Germany) or 3.0 Tesla Siemens TIM Trio (Erlangen, Germany) scanner. For the Siemens Biograph mMR, high-resolution 3-dimensional sagittal T1 magnetization prepared – rapid gradient echo (MP-RAGE) anatomical images were acquired with the scanning parameters of repetition time (TR) = 2300 ms, time to echo (TE) = 2.95 ms, flip angle = 9°, 176 slices, acquisition matrix = 240 × 256, and voxel size = 1 × 1 × 1.2 mm^3. For the Siemens TIM Trio, high-resolution 3-dimensional sagittal T1 MP-RAGE anatomical images were acquired with the scanning parameters of TR = 2400 ms, TE = 3.16 ms, flip angle = 8°, 176 slices, acquisition matrix = 256 × 256, and voxel size = 1 × 1 × 1 mm^3. Total gray and white matter volumes were extracted from T1

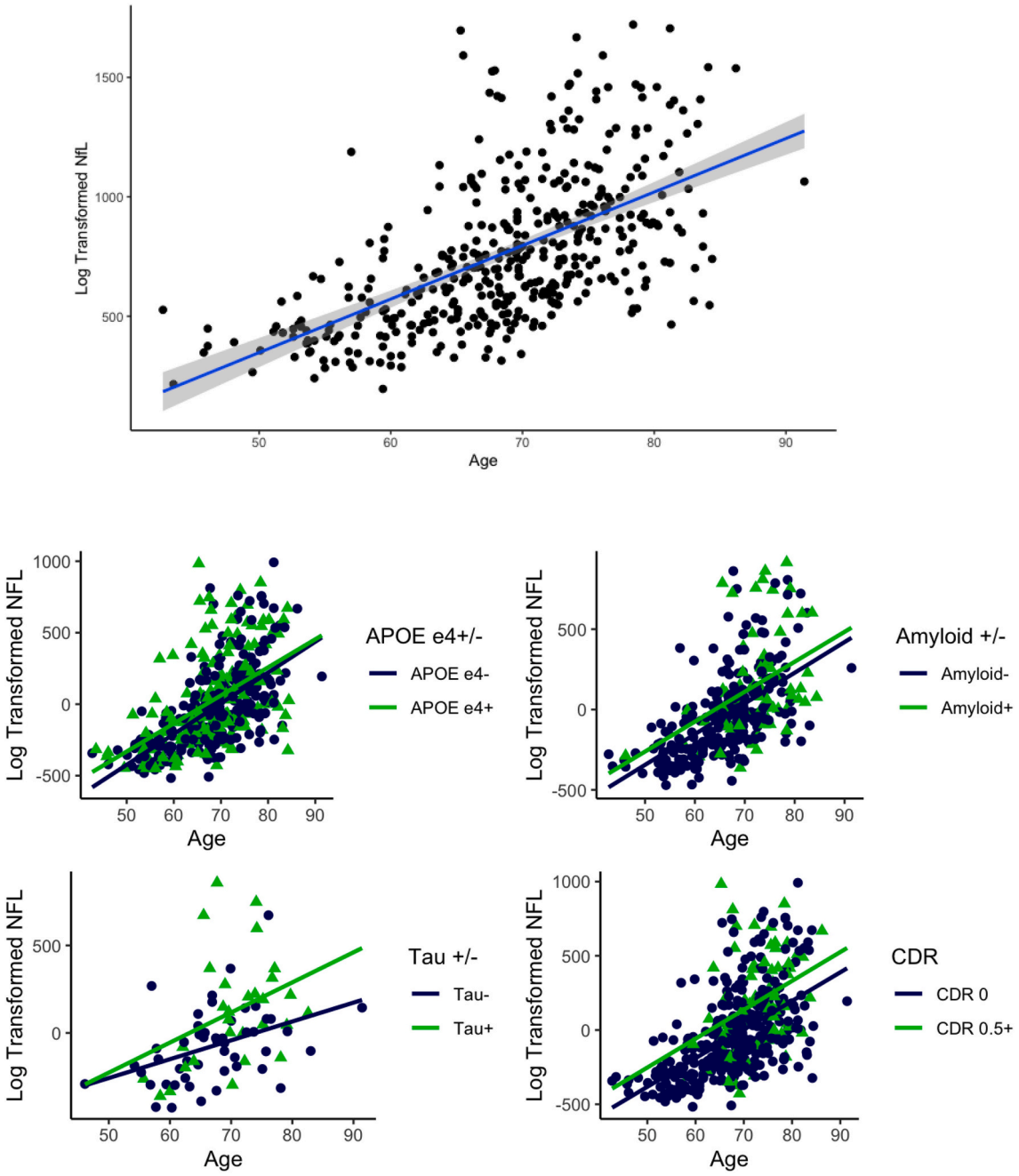

Fig. 1. A positive relationship was observed between cerebrospinal fluid (CSF) neurofilament light chain (NfL) and age. A: The exponential relationship between NfL and age. B: Data points are color coded by *APOE ε4* status C: Data points are color coded by amyloid PET status. D: Data points are color coded by tau PET status. E: Data points are color coded by Clinical Dementia Rating (CDR). B-E: scatterplots represent associations controlling for sex and race. CSF NfL values were log-transformed, centered and scaled due to skewed distributions.

structural MRI and were segmented using FreeSurfer 5.3 (http://freesurfer.net). Total gray matter volume was calculated by taking the sum of cortical and subcortical gray matter regions for the left and right hemispheres, whereas total white matter volume was calculated as the sum of the total left and right cortical white matter regions. Total WMH volume was extracted from FLAIR scans using the lesion segmentation toolbox (LST) (Schmidt et al., 2012) implemented in SPM8.

2.1.5. Positron emission tomography imaging

Amyloid burden was determined using PET [^{11}C] Pittsburgh compound B (PiB) or florbetapir (Kuhle et al., 2019) F-AV-45). Participants received a single intravenous bolus of PiB or (Kuhle et al., 2019) F-AV-45 infused for 20 s. Three-dimensional axial PET images were acquired using a Siemens Biograph 3.0 Tesla mMR with the scanning parameters of 176 slices, acquisition matrix = 256 × 256, and voxel size = 1.12 × 1.12 × 2.03 mm^3. Attenuation correction was performed using computed tomography (CT). Data from 30 to 60 min for PiB or 50–70 min for (Kuhle et al., 2019) F-AV-45 post injection were converted to standard uptake value ratios (SUVR) with the cerebellar cortex serving as the reference region. An in-house PET unified pipeline (PUP) was used to process PET images (Su et al., 2013; Su et al., 2015). SUVRs from the lateral orbitofrontal, medial orbitofrontal, middle temporal, precuneus, rostral middle frontal, superior frontal, and superior temporal cortices (defined by FreeSurfer) were averaged to define the mean cortical amyloid SUVR. To standardize across PiB and (Kuhle et al., 2019) F-AV-45, SUVRs were converted to Centiloids (Klunk et al., 2015;

Table 2
Multiple regression results of age and AD marker status predicting cerebrospinal fluid (CSF) neurofilament light (NfL) with age, sex, and race as covariates.

APOE ε4 status				Tau status			
	t	β (SE)	*p*		*t*	β (SE)	*p*
APOE ε4	2.14	0.08 (25.08)	0.03	Tau	2.56	0.24 (58.69)	0.01
Age	14.25	0.56 (1.50)	<0.001	Age	3.55	0.35 (3.82)	<0.001
Sex	−4.75	−0.18 (24.78)	<0.001	Sex	−3.45	−0.33 (58.64)	<0.001
Race	−2.38	−0.09 (44.66)	0.02	Race	−2.07	−0.20 (130.88)	0.04
Amyloid status				CDR Status			
Amyloid	2.23	0.11 (34.53)	0.03	CDR	4.23	0.16 (32.72)	<0.001
Age	11.24	0.54 (1.72)	<0.001	Age	13.34	0.52 (1.50)	<0.001
Sex	−3.82	−0.17 (27.51)	<0.001	Sex	−4.50	−0.17 (24.30)	<0.001
Race	−0.62	−0.03 (46.71)	0.54	Race	−2.14	−0.08 (43.63)	0.03

Abbreviations: APOE ε4 = apolipoprotein ε4 allele; CDR = Clinical Dementia Rating Scale.

Table 3
Multiple regression results of gray, white, and white matter hyperintensity (WMH) volumes predicting cerebrospinal fluid (CSF) neurofilament light (NfL). Age, sex, race, and total intracranial volume (ICV) were included as covariates.

WMH Volume			
	t	β (SE)	*p*
WMH	4.32	0.24 (16.77)	<0.001
Age	8.59	0.42 (1.84)	<0.001
Sex	−4.15	−0.22 (32.08)	<0.001
Race	−2.34	−0.10 (45.70)	0.02
ICV	−1.19	−0.07 (0.00)	0.23
Gray matter volume			
Gray Matter	−2.38	−0.15 (20.70)	0.02
Age	9.90	0.48 (1.86)	<0.001
Sex	−2.94	−0.15 (32.00)	<0.001
Race	−2.25	−0.09 (45.20)	0.03
ICV	2.83	0.19 (0.00)	<0.001
White matter volume			
	t	β (SE)	*p*
White Matter	−1.23	−0.08 (20.79)	0.22
Age	10.83	0.51 (1.82)	<0.001
Sex	−2.66	−0.13 (32.01)	0.01
Race	−1.90	−0.07 (45.15)	0.06
ICV	2.18	0.15 (0.00)	0.03
WMH + Gray + White Matter Volumes			
WMH	3.94	0.22 (17.25)	<0.001
Gray Matter	−1.27	−0.10 (23.77)	0.20
White Matter	−0.10	−0.01 (22.45)	0.92
Age	6.76	0.38 (2.14)	<0.001
Sex	−4.13	−0.22 (32.27)	<0.001
Race	−2.46	−0.10 (46.47)	0.01
ICV	0.30	0.03 (0.00)	0.77

Su et al., 2018). Briefly, the Centiloid scale is defined by two anchor points: the mean amyloid burden of a young control group, and the mean amyloid burden of an AD group. The mean amyloid burden of the AD group was represented as 100 in the Centiloid scale. Regression and linear transformation were performed to calibrate the tracers and local processing methods to the Centiloid scale (Klunk et al., 2015). Amyloid positivity was subsequently defined as having a Centiloid value of 16.4 or greater.

PET tau imaging was performed using [18F]-Flortaucipir (AV1451), acquired on a Biograph 40 PET/CT scanner (Siemens Medical Solutions) with SUVRs calculated for the 80–100-min post-injection window. A summary measure of tauopathy, previously defined as the mean of the amygdala, entorhinal cortex, inferior temporal region, and lateral occipital regions based on FreeSurfer 5.3 segmentation, was calculated for each participant. Tau positivity was defined as having an AV1451 SUVR value of 1.22 or greater (Mishra et al., 2017).

2.2. Statistical analysis

CSF NfL values were skewed and were transformed with the natural logarithm for all statistical analyses. Similarly, all values for total gray, white, and WMH volume were log-transformed, centered and scaled due to skewed distributions. An analysis of covariance (ANCOVA) was used for preliminary comparisons of CSF NfL levels between groups defined by race (Black or Non-Hispanic White) and sex, controlling for age and education.

Pearson's (r) correlation was used to assess the relationship between CSF NfL and age. Four multiple regression models were used to examine the relationship between CSF NfL and categorical AD marker status (i.e., amyloid, tau, CDR, or *APOE* ε4 status). For each model, CSF NfL was the outcome variable and AD marker status was the respective predictor variable. Age, sex, and race were included as covariates in all four models.

Three multiple regression models were performed to determine whether brain volumes (i.e., total gray matter, white matter, or WMH volume) were associated with CSF NfL. For all three models, CSF NfL was the outcome variable and respective brain volume was the predictor. Age, sex, race, and intracranial volume (ICV) were included as covariates in the models. To determine which brain volume (i.e., gray, white, or WMH) most strongly predicted CSF NfL, we conducted a single multiple regression model with CSF NfL as the outcome variable and with brain volumes simultaneously entered as predictor variables. Age, sex, race, and ICV were included as covariates in the model.

After examining the initial associations between CSF NfL, WMH, and AD markers, four exploratory stepwise regression models (forward and backwards) with dominance analysis were used to determine which independent variables (i.e., age, WMH, AD marker status) are the strongest and most *important* predictors of CSF NfL. For each model, CSF NfL was the outcome variable and age, WMH volume and AD marker status were the respective predictor variables. Sex, race, and ICV were also included as available predictor variables for selection in all models. For the stepwise regressions, Akaike information criterion (AIC) and *p*-values (significance level at $p < 0.05$) were used to distinguish the best-fit model based on the available predictors. AIC uses maximum likelihood estimates and the number of parameters (i.e., predictor variables) to determine the relative information value of the model. The formula for AIC is AIC = 2 k-2ln(L) where k is the number of predictor variables and L is the log-likelihood estimate. The default K is always 2, so if the model uses one predictor variable, $K = 3$. For each model, there were 6 available predictors (i.e., age, sex, race, WMH volume, ICV, and the respective marker), yielding a K of 7. If a model is more than 2 AIC units lower than another, it is considered significantly better. Only best-fit and significant predictors were included in the final models. The strength of each individual predictor was interpreted using β values.

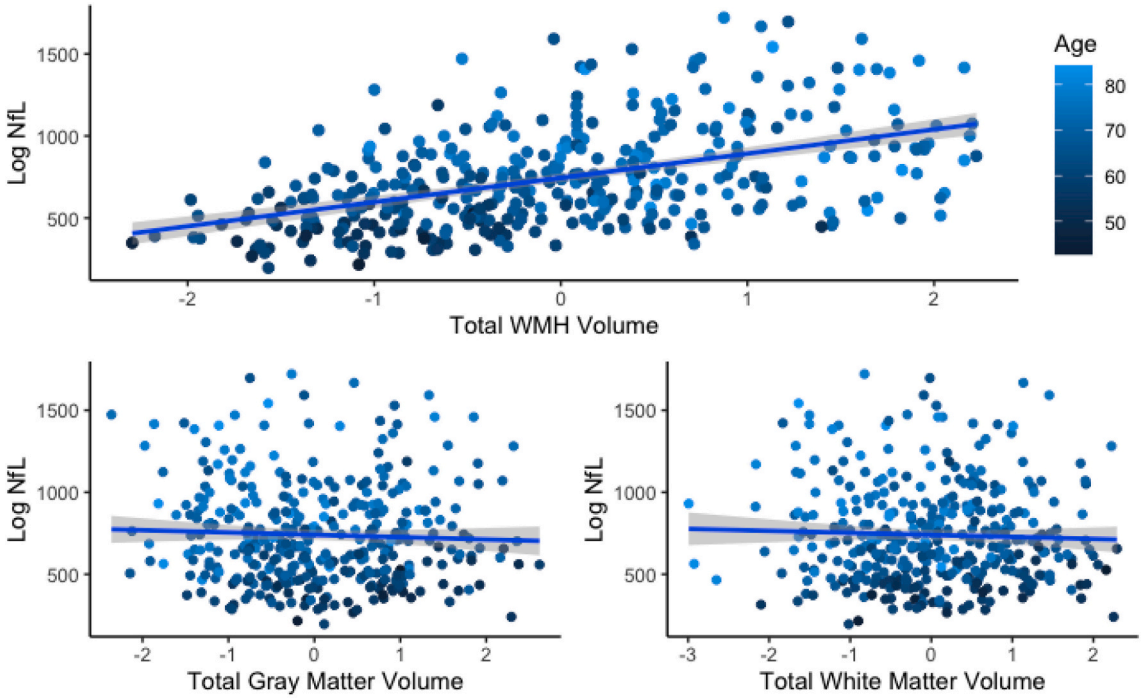

Fig. 2. Associations between CSF NfL and total white matter hyperintensities (WMH), gray, and white matter volumes. Plots reflect the association after controlling for age, race, and sex. Dots are color coded by age. Gray shading represents the 95% confidence interval. CSF NfL, total gray, white, and WMH volume values were log-transformed, centered and scaled due to skewed distributions.

Dominance analysis is an informative and straightforward statistical approach that determines the relative importance (i.e., dominance) of a predictor over another by comparing the incremental R^2 contribution across all possible subset models. Importance is determined in a pairwise fashion where the respective pair of predictors are compared in all $2^{(p-2)}$ (p = # of predictors) that contain some subset of the other predictors (Azen and Budescu, 2003). Dominance can be achieved at 3 levels including complete, conditional, and general dominance. Complete dominance occurs when one predictor's dominance is maintained across all possible subset models, excluding the two predictors under comparison. Complete dominance implies conditional and general dominance and is the primary focus of these analyses. All statistical tests were conducted in RStudio (version 1.2.5042).

3. Results

3.1. CSF NfL as function of sex, race, APOE ε4, Amyloid PET, Tau PET, and CDR status

CSF NfL was higher in men compared to women ($p < 0.001$) and in Non-Hispanic Whites compared to Blacks ($p < 0.001$) after controlling for age and education. See Table 1 for participant characteristics by CDR and PET status. Because education was not a significant predictor in any of the models, it was not used as a covariate in subsequent analyses. The full ANCOVA model can be found in Supplemental Materials Table 1.

3.2. CSF NfL increases with age and is higher in individuals with markers of AD

CSF NfL was positively correlated with age ($r = 0.59$, $p < 0.001$; see Fig. 1A). Results from the multiple regression models examining whether NfL was associated with AD marker status demonstrated that there was a significant effect for age, *APOE* ε4 status, amyloid PET status, tau PET status, and cognitive status (all p's < 0.05; see Table 2). Specifically, *APOE* ε4+, amyloid+, tau+, or cognitively impaired (CDR > 0) individuals had higher CSF NfL compared to AD marker negative individuals. All analyses were repeated with continuous measures of amyloid PET, tau PET, and cognition (i.e., CDR-SB and MMSE) and yielded a similar pattern of results (see Supplemental Materials Table 2). Associations between CSF NfL and age by AD marker status are shown in Fig. 1.

3.3. CSF NfL associates more with WMH volume than total gray and white matter volumes

Results from the individual multiple regression analyses demonstrated that across the entire cohort, total gray ($\beta = -0.15$, $p < 0.05$) and WMH volumes ($\beta = 0.24$, $p < 0.001$) significantly predicted CSF NfL when age, sex, race, and ICV were considered, while total white matter volume was not significant ($\beta = -0.08$, $p > 0.05$; see Table 3). Specifically, higher CSF NfL was associated with reduced gray matter volume and greater WMH volume.

When all three volumes were simultaneously entered into the model with age, sex, race, and ICV included as covariates, only WMH volume significantly predicted CSF NfL ($\beta = 0.22$, $p < 0.001$). Total gray and white matter volumes were not significantly associated with CSF NfL (p's > 0.05; see Table 3 and Fig. 2). Higher CSF NfL was associated with greater WMH volume.

3.4. Age and WMH volume are the most important predictors of CSF NfL

Stepwise (forward and backwards) regression models indicated that when considering associations between CSF NfL and age, sex, race, WMH volume, and AD marker status (i.e., amyloid, tau, CDR, or *APOE* ε4 status), age consistently demonstrated the strongest relationship with CSF NfL (all p's < 0.001), followed by WMH volume and sex (all p's < 0.01; see Table 4 for β values and final models). Additional effects of race, *APOE* ε4 status, amyloid status, and CDR status were observed across the models (all p's < 0.05), albeit to a lesser extent than age and sex. The stepwise regressions did not identify ICV or tau status as significant predictors of CSF NfL and were therefore excluded from final models. All analyses were repeated with continuous measures of

Table 4
Stepwise regression and dominance analysis results of age, sex, race, brain volume, and AD marker status predicting cerebrospinal fluid (CSF) neurofilament light (NfL).

APOE ε4 status						
Final Model	R^2	R^2 Adj.	*t*	β (SE)	AIC	*p*
Age	0.326	0.324	9.15	0.44 (1.79)	5231.84	<0.001
WMH	0.367	0.363	4.22	0.20 (14.65)	5210.36	<0.001
Sex	0.395	0.390	−4.31	−0.18 (24.76)	5195.27	<0.001
Race	0.402	0.396	−2.23	−0.09 (44.89)	5192.63	0.03
APOE	0.409	0.401	2.04	0.08 (24.81)	5190.44	0.04
Amyloid status						
Age	0.352	0.349	7.71	0.45 (2.11)	3676.65	<0.001
Sex	0.394	0.390	−3.61	−0.17 (27.90)	3859.75	<0.001
WMH	0.408	0.402	2.98	0.17 (17.46)	3855.08	<0.01
Amyloid	0.425	0.416	2.82	0.14 (34.85)	3849.12	<0.01
Tau status						
Age	0.228	0.215	4.07	0.43 (4.14)	905.97	<0.001
Sex	0.319	0.297	−2.89	−0.31 (59.42)	899.76	<0.01
CDR status						
Age	0.326	0.324	8.84	0.42 (1.77)	5231.84	<0.001
WMH	0.367	0.363	3.71	0.18 (14.60)	5210.36	<0.001
Sex	0.395	0.390	−4.39	−0.18 (24.40)	5195.27	<0.001
CDR	0.419	0.413	3.92	0.16 (35.03)	5181.53	<0.001
Race	0.426	0.418	−2.03	−0.08 (44.22)	5179.36	0.04

Note: Only final, best-fit models with significant predictors are reported; non-significant predictors were removed from the final models. Akaike information criterion (AIC) and p-values (significance level at $p < 0.05$) were used to distinguish the best-fit model based on the available predictors. Abbreviations: APOE ε4 = apolipoprotein ε4 allele; WMH = white matter hyperintensity volume; CDR = Clinical Dementia Rating Scale.

amyloid PET, tau PET, and cognition (i.e., CDR-SB and MMSE) and yielded a similar pattern of results (see Supplemental Materials Table 3). Dominance analyses additionally demonstrated that in every model predicting CSF NfL, age was the most important predictor, as indicated by R^2 values, followed by WMH volume and sex (see Fig. 3). For every model, age held *complete* dominance over the other variables. Furthermore, WMH always held complete dominance over every AD status marker, including *APOE* ε4 status (Fig. 3A), amyloid status (Fig. 3B), tau status (Fig. 3C), or CDR (Fig. 3D). Together this highlights the maintained importance of age and WMH over AD status markers.

4. Discussion

In the present study, we examined the relationship between CSF NfL, age, and markers of AD marker status. We further examined the neuroanatomical basis for elevated NfL in terms of its association with total gray, white, or WMH volumes and determined the relative importance of AD maker status when predicting CSF NfL. Results show that CSF NfL increases linearly and is associated with AD status markers. Furthermore, CSF NfL was associated with total WMH volume, but not gray or white matter volumes when all volumes were considered simultaneously. When considering age, AD marker status, and WMH volume jointly, age was consistently the strongest and most important predictor of NfL, followed by WMH. Collectively these results suggest that elevated CSF NfL is a marker of aging (i.e., the multiple unmeasured changes related to age and age-related comorbidities, such as increased risk for hypertension, diabetes, and cerebrovascular disease) and white matter integrity and has less specificity for AD processes.

This study compared the association between CSF NfL and total gray, white, and WMH volume. Previous research has demonstrated that elevated CSF NfL is independently associated with hippocampal atrophy (Mattsson et al., 2017; Zetterberg et al., 2016; Idland et al., 2017), cortical thinning (Mattsson et al., 2017; Preische et al., 2019; Pereira et al., 2017), larger ventricular volume (Mattsson et al., 2017; Zetterberg et al., 2016), and faster accumulation of WMHs[10]. Elevated CSF NfL has also been associated with WMH volume (Duering et al., 2018; Sjögren et al., 2001; Jonsson et al., 2010) and lacunar infarct volume (Duering et al., 2018). The present study, which simultaneously tested associations between brain volumes, demonstrated that CSF NfL was only associated with WMH volume relative to gray and white matter volume. NfL is an axonal structural protein (Petzold, 2005), therefore it is unsurprising that it is most strongly associated with WMHs. WMHs reflect demyelination and axonal loss and are the consequence of small vessel disease (SVD) and cerebrovascular health (Pantoni and Garcia, 1997; Lazarus et al., 2005; Prins and Scheltens, 2015; Debette and Markus, 2010). Our results support the hypothesis that CSF NfL is a marker of cumulative SVD (Korley et al., 2019; Duering et al., 2018; Jonsson et al., 2010), and further show that this process is likely independent of amyloid and tau.

The finding that CSF NfL is associated with amyloid and tau PET is in concordance with several studies demonstrating associations with markers of AD (Mattsson et al., 2017; Zetterberg et al., 2016; Preische et al., 2019; Zhou et al., 2017; Jin et al., 2019; Olsson et al., 2016). For example, Mattsson and colleagues (2017) (Mattsson et al., 2017) demonstrated that in individuals with mild cognitive impairment (MCI), plasma NfL is significantly associated with CSF Aβ42 and total-tau. Zetterberg and colleagues (2016) (Zetterberg et al., 2016) reported a similar association between CSF NfL and Aβ42; however, this relationship was only significant across the entire sample (i.e., cognitively normal, MCI, and AD) and did not differ by diagnostic status, thus suggesting a lack of sensitivity to AD processes or lack of adequate power.

Despite evidence that CSF NfL was associated with age, WHH, and markers of AD status, the inter-relationship between these diverse factors in predicting CSF NfL levels has remained unclear. To address this issue, we performed exploratory stepwise regressions with dominance analyses to more fully investigate the precise contributions of age and AD markers in their associations with CSF NfL, while continuing to include the contribution of WMH volumes. Results indicated that although there were significant associations between CSF NfL and *APOE* ε4 status, amyloid status, and CDR status, the effects were relatively small as compared to age (see Table 4 and Supplementary Materials Table 3). Furthermore, age and WMH volumes were consistently the most important of the predictors (see Fig. 3). We further demonstrated that age largely attenuates the association between CSF NfL and amyloid PET and tau PET (Supplemental Materials Fig. 1). Collectively, these results along with the documented associations between CSF NfL and WMH support the hypothesis that CSF NfL is a marker of aging (and in particular, cumulative SVD burden) rather than being a specific marker of AD-related pathology.

This study has several limitations. Subgroups were biased based on available data, with comparatively fewer individuals with tau PET compared to amyloid PET. The number of individuals with more advanced AD was also limited, therefore, interpretations of the present findings are mostly focused on preclinical and very early symptomatic stages of AD and do not necessarily apply to the later stages of AD or to individuals with other disease etiologies. Future studies should also be conducted using a cohort with more advanced AD. Furthermore, it remains unclear whether WMH in different brain regions (e.g., periventricular, juxtacortical, deep white matter, etc.) or in specific white matter tracts result in similar elevations in NfL. Future studies are needed to examine parcellations of white matter and its relationship to CSF NfL.

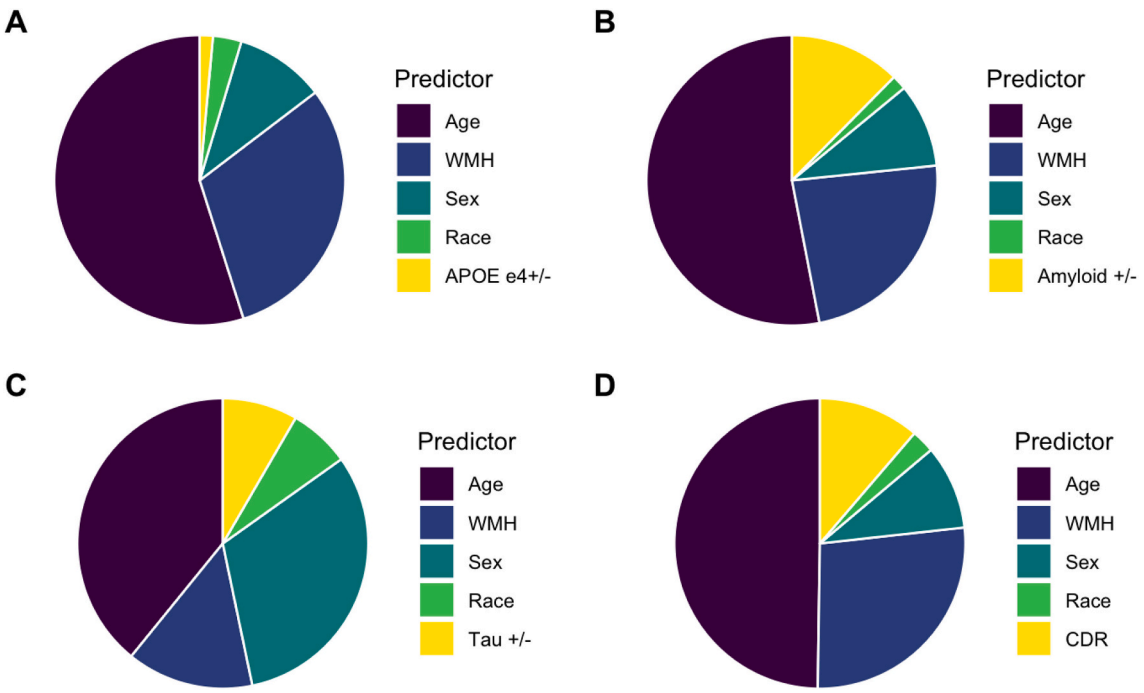

Fig. 3. Relative importance of age, white matter hyperintensity (WMH) volume, sex, race, and AD marker status in predicting cerebrospinal fluid (CSF) neurofilament light (NfL). Shaded areas represent R2 values from dominance analyses. Models vary only in the predictor used for AD marker status, with A: APOE ε4 status, B: amyloid status, C: tau status, or D: Clinical Dementia Rating (CDR) scale status.

5. Conclusions

Overall, this study suggests that CSF NfL levels reflect an amyloid-independent mechanism of aging. CSF NfL levels largely reflect aging and cumulative small vessel disease burden as assayed by relative WMH volume rather AD-specific measures.

Author contributions

Dr. Meeker had full access to all the data and takes responsibility for the integrity of the data and accuracy of the data analysis.

Karin Meeker: conceptualization, methodology, software, formal analysis, data curation, writing- original draft, writing-review and editing, visualization. Omar Butt: writing-original draft, writing-review and editing. Brian Gordon: conceptualization, methodology, writing-review and editing. Suzanne Schindler: resources, writing-review and editing, funding acquisition. John Morris: resources, writing-review and editing, funding acquisition. Anne Fagan: resources, writing-review and editing. Tammie Benzinger: resources, writing-review and editing. Beau Ances: conceptualization, methodology, resources, writing-review and editing, supervision, revising, funding acquisition.

Role of the Funder/Sponsor

No sponsor had any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional contributions

The authors thank the participants, investigators, and staff of the Knight Alzheimer Disease Research Center (ADRC) Clinical Core for participant assessments, Genetics Core for *APOE* ε genotyping, Fluid Biomarker Core for CSF biomarker analyses, and the Imaging Core for amyloid, tau, and structural imaging.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2022.105662.

References

Azen, R., Budescu, D.V., 2003. The dominance analysis approach for comparing predictors in multiple regression. Psychol. Methods 8 (2), 129–148. https://doi.org/10.1037/1082-989X.8.2.129.

Bäckström, D.C., Domellöf, M.E., Linder, J., et al., 2015. Cerebrospinal fluid patterns and the risk of future dementia in early, incident Parkinson disease. JAMA Neurol. 72 (10), 1175–1182. https://doi.org/10.1001/jamaneurol.2015.1449.

Bergman, J., Dring, A., Zetterberg, H., et al., 2016. Neurofilament light in CSF and serum is a sensitive marker for axonal white matter injury in MS. Neurol Neuroimmunol neuroinflammation. 3 (5), e271 https://doi.org/10.1212/NXI.0000000000000271.
Buckner, R.L., Sepulcre, J., Talukdar, T., et al., 2009. Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer disease. J. Neurosci. 29 (6), 1860–1873. https://doi.org/10.1523/JNEUROSCI.5062-08.2009.
Cruchaga, C., Kauwe, J.S.K., Harari, O., et al., 2013. GWAS of cerebrospinal fluid tau levels identifies risk variants for alzheimer disease. Neuron. 78 (2), 256–268. https://doi.org/10.1016/j.neuron.2013.02.026.
Debette, S., Markus, H.S., 2010. The clinical importance of white matter hyperintensities on brain magnetic resonance imaging: systematic review and meta-analysis. BMJ. 341 (7767), 288. https://doi.org/10.1136/bmj.c3666.
Duering, M., Konieczny, M.J., Tiedt, S., et al., 2018. Serum neurofilament light chain levels are related to small vessel disease burden. J Stroke. 20 (2), 228–238. https://doi.org/10.5853/jos.2017.02565.
Eikelenboom, M.J., Petzold, A., Lazeron, R.H.C., et al., 2003. Multiple sclerosis: Neurofilament light chain antibodies are correlated to cerebral atrophy. Neurology. 60 (2), 219–223. https://doi.org/10.1212/01.wnl.0000041496.58127.e3.
Fagan, A.M., Mintun, M.A., Mach, R.H., et al., 2006. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid Aβ 42 in humans. Ann. Neurol. 59 (3), 512–519. https://doi.org/10.1002/ana.20730.
Gaetani, L., Blennow, K., Calabresi, P., Di, Filippo M., Parnetti, L., Zetterberg, H., 2019. Neurofilament light chain as a biomarker in neurological disorders neurodegeneration. J. Neurol. Neurosurg. Psychiatry 90, 870–881. https://doi.org/10.1136/jnnp-2018-320106.
Gaiottino, J., Norgren, N., Dobson, R., et al., 2013. In: Reindl, M. (Ed.), Increased Neurofilament Light Chain Blood Levels in Neurodegenerative Neurological Diseases, 8(9). PLoS One. https://doi.org/10.1371/journal.pone.0075091 e75091.
Gordon, B.A., 2020. Neurofilaments in disease: what do we know? Curr. Opin. Neurobiol. 61, 105–115. https://doi.org/10.1016/j.conb.2020.02.001.
Henson, R.L., Doran, E., Christian, B.T., et al., 2020. Cerebrospinal fluid biomarkers of Alzheimer disease in a cohort of adults with down syndrome. Alzheimer's Dement Diagnosis, Assess Dis Monit. 12 (1) https://doi.org/10.1002/dad2.12057.
Idland, A.V., Sala-Llonch, R., Borza, T., et al., 2017. CSF neurofilament light levels predict hippocampal atrophy in cognitively healthy older adults. Neurobiol. Aging 49, 138–144. https://doi.org/10.1016/j.neurobiolaging.2016.09.012.
Jin, M., Cao, L., Dai, Y.P., 2019. Role of Neurofilament light chain as a potential biomarker for Alzheimer disease: a correlative Meta-analysis. Front. Aging Neurosci. 11, 254. https://doi.org/10.3389/fnagi.2019.00254.
Jonsson, M., Zetterberg, H., Van Straaten, E., et al., 2010. Cerebrospinal fluid biomarkers of white matter lesions - cross-sectional results from the LADIS study. Eur. J. Neurol. 17 (3), 377–382. https://doi.org/10.1111/j.1468-1331.2009.02808.x.
Khalil, M., Teunissen, C.E., Otto, M., et al., 2018. Neurofilaments as biomarkers in neurological disorders. Nat. Rev. Neurol. 14 (10), 577–589. https://doi.org/10.1038/s41582-018-0058-z.
Klunk, W.E., Koeppe, R.A., Price, J.C., et al., 2015. The Centiloid project: standardizing quantitative amyloid plaque estimation by PET. Alzheimers Dement. 11 (1), 1–15 e4. https://doi.org/10.1016/j.jalz.2014.07.003.
Korley, F.K., Goldstick, J., Mastali, M., et al., 2019. Serum NfL (Neurofilament light chain) levels and incident stroke in adults with diabetes mellitus. Stroke. 50 (7), 1669–1675. https://doi.org/10.1161/STROKEAHA.119.024941.
Kuhle, J., Kropshofer, H., Haering, D.A., et al., 2019. Blood neurofilament light chain as a biomarker of MS disease activity and treatment response. Neurology. 92 (10), E1007–E1015. https://doi.org/10.1212/WNL.0000000000007032.
Landqvist Waldö, M., Frizell Santillo, A., Passant, U., et al., 2013. Cerebrospinal fluid neurofilament light chain protein levels in subtypes of frontotemporal dementia. BMC Neurol. 13 (1), 1–8. https://doi.org/10.1186/1471-2377-13-54.
Lazarus, R., Prettyman, R., Cherryman, G., 2005. White matter lesions on magnetic resonance imaging and their relationship with vascular risk factors in memory clinic attenders. Int J Geriatr Psychiatry. 20 (3), 274–279. https://doi.org/10.1002/gps.1283.
Lu, C.H., Macdonald-Wallis, C., Gray, E., et al., 2015. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology. 84 (22), 2247–2257. https://doi.org/10.1212/WNL.0000000000001642.
Mattsson, N., Insel, P.S., Palmqvist, S., et al., 2016. Cerebrospinal fluid tau, neurogranin, and neurofilament light in Alzheimer disease. EMBO Mol Med. 8, 1184–1196. https://doi.org/10.15252/emmm.201606540.
Mattsson, N., Andreasson, U., Zetterberg, H., Blennow, K., 2017. Association of Plasma Neurofilament Light with Neurodegeneration in patients with Alzheimer disease. JAMA Neurol. 74 (5), 557. https://doi.org/10.1001/jamaneurol.2016.6117.
McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer disease. Neurology. 34 (7), 939. https://doi.org/10.1212/WNL.34.7.939.
Mishra, S., Gordon, B.A., Su, Y., et al., 2017. AV-1451 PET imaging of tau pathology in preclinical Alzheimer disease: defining a summary measure. Neuroimage. 161, 171–178. https://doi.org/10.1016/j.neuroimage.2017.07.050.
Morris, J.C., 1993. The clinical dementia rating (CDR): current version and scoring rules. Neurology. 43 (11), 2412. https://doi.org/10.1212/WNL.43.11.2412-a.
Olsson, B., Lautner, R., Andreasson, U., et al., 2016. CSF and blood biomarkers for the diagnosis of Alzheimer disease: a systematic review and meta-analysis. Lancet Neurol. 15 (7), 673–684. https://doi.org/10.1016/S1474-4422(16)00070-3.
Pantoni, L., Garcia, J.H., 1997. Pathogenesis of Leukoaraiosis. Stroke. 28 (3), 652–659. https://doi.org/10.1161/01.STR.28.3.652.
Pereira, J.B., Westman, E., Hansson, O., 2017. Association between cerebrospinal fluid and plasma neurodegeneration biomarkers with brain atrophy in Alzheimer disease. Neurobiol. Aging 58, 14–29. https://doi.org/10.1016/J.NEUROBIOLAGING.2017.06.002.
Petzold, A., 2005. Neurofilament phosphoforms: Surrogate markers for axonal injury, degeneration and loss. In: Journal of the Neurological Sciences, Vol 233. Elsevier, pp. 183–198. https://doi.org/10.1016/j.jns.2005.03.015.
Petzold, A., Keir, G., Warren, J., Fox, N., Rossor, M.N., 2007. A systematic review and meta-analysis of CSF neurofilament protein levels as biomarkers in dementia. Neurodegener. Dis. 4 (2–3), 185–194. https://doi.org/10.1159/000101843.
Preische, O., Schultz, S.A., Apel, A., et al., 2019. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer disease. Nat. Med. 25 (2), 277–283. https://doi.org/10.1038/s41591-018-0304-3.
Prins, N.D., Scheltens, P., 2015. White matter hyperintensities, cognitive impairment and dementia: an update. Nat. Rev. Neurol. 11 (3), 157–165. https://doi.org/10.1038/nrneurol.2015.10.
Schmidt, P., Gaser, C., Arsic, M., et al., 2012. An automated tool for detection of FLAIR-hyperintense white-matter lesions in multiple sclerosis. Neuroimage. 59 (4), 3774–3783. https://doi.org/10.1016/j.neuroimage.2011.11.032.
Sjögren, M., Blomberg, M., Jonsson, M., et al., 2001. Neurofilament protein in cerebrospinal fluid: a marker of white matter changes. J. Neurosci. Res. 66 (3), 510–516. https://doi.org/10.1002/jnr.1242.
Skillbäck, T., Farahmand, B., Bartlett, J.W., et al., 2014. CSF neurofilament light differs in neurodegenerative diseases and predicts severity and survival. Neurology. 83 (21), 1945–1953. https://doi.org/10.1212/WNL.0000000000001015.
Sperling, R.A., LaViolette, P.S., O'Keefe, K., et al., 2009. Amyloid deposition is associated with impaired default network function in older persons without dementia. Neuron. 63 (2), 178–188. https://doi.org/10.1016/J.NEURON.2009.07.003.
Su, Y., D'Angelo, G.M., Vlassenko, A.G., et al., 2013. In: Chen, K. (Ed.), Quantitative analysis of PiB-PET with FreeSurfer ROIs, 8(11). PLoS One. https://doi.org/10.1371/journal.pone.0073377 e73377.
Su, Y., Blazey, T.M., Snyder, A.Z., et al., 2015. Partial volume correction in quantitative amyloid imaging. Neuroimage. 107, 55–64. https://doi.org/10.1016/j.neuroimage.2014.11.058.
Su, Y., Flores, S., Hornbeck, R.C., et al., 2018. Utilizing the Centiloid scale in cross-sectional and longitudinal PiB PET studies. NeuroImage Clin. 19, 406–416. https://doi.org/10.1016/j.nicl.2018.04.022.
Yuan, A., Rao, M.V., Veeranna, Nixon R.A., 2017. Neurofilaments and neurofilament proteins in health and disease. Cold Spring Harb. Perspect. Biol. 9 (4), a018309 https://doi.org/10.1101/cshperspect.a018309.
Zetterberg, H., Skillbäck, T., Mattsson, N., et al., 2016. Association of Cerebrospinal Fluid Neurofilament Light Concentration with Alzheimer Disease Progression. JAMA Neurol. 73 (1), 60. https://doi.org/10.1001/jamaneurol.2015.3037.
Zhou, W., Zhang, J., Ye, F., et al., 2017. Plasma neurofilament light chain levels in Alzheimer disease. Neurosci. Lett. 650, 60–64. https://doi.org/10.1016/J.NEULET.2017.04.027.