Neurobiology of Aging 141 (2024) 74–84

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging.org

Age-adjusted CSF t-tau and NfL do not improve diagnostic accuracy for prodromal Alzheimer's disease

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ARTICLE INFO

Keywords:
Alzheimer's disease
aging
total-tau
neurofilament light chain
normative reference data

ABSTRACT

Cerebrospinal fluid total-tau (t-tau) and neurofilament light chain (NfL) are biomarkers of neurodegeneration and are increased in Alzheimer's disease (AD). In order to adjust for age-related increases in t-tau and NfL, cross-sectional age-adjusted norms were developed based on amyloid negative cognitively normal (CN) adults aged 41–78 years (CN, n = 137). The age-adjusted norms for t-tau and NfL did not improve receiver operating curve based diagnostic accuracies in individuals with mild cognitive impairment (MCI) due to AD (AD-MCI, n = 144). Furthermore, while NfL was correlated with higher age in AD-MCI, no significant correlation was found for t-tau. The cox proportional hazard models, applied in 429 participants with baseline t-tau and NfL, showed higher hazard ratio of progression to MCI or dementia without age-adjustments (HR = 3.39 for t-tau and HR = 3.17 for NfL), as compared to using our norms (HR = 2.29 for t-tau and HR = 1.89 for NfL). Our results indicate that utilizing normative reference data could obscure significant age-related increases in these markers associated with neurodegeneration and AD leading to a potential loss of overall diagnostic accuracy.

1. Background

The neuropathological hallmarks of Alzheimer's disease (AD) are amyloid plaques (Aβ) and neurofibrillary tangles followed by downstream neurodegeneration, which can be measured with cerebrospinal fluid (CSF) biomarkers (De Strooper and Karran, 2016). Total-tau (t-tau) is a non-specific marker of neuronal and axonal injury (Zetterberg, 2017, Blennow and Hampel, 2003). In AD, CSF t-tau are stably increased over years (Zetterberg et al., 2007), and previous studies have shown a positive correlation between increasing age and higher concentrations of CSF t-tau also in clinically healthy adults (Shoji et al., 2002, Sjogren et al., 2001, Mattsson et al., 2009, Zebhauser et al., 2021). Despite this, general t-tau cut-offs are still most common (Zebhauser et al., 2021), even though age-adjusted cut-offs have been suggested (Sjogren et al., 2001). Neurofilament light chain (NfL) is also a non-specific, but sensitive CSF and plasma biomarker for neuroaxonal injury (Vågberg et al., 2015). Evidence suggests that increased NfL concentrations in AD are independent of amyloid beta (Aβ) load (Mattsson et al., 2016, Skillbäck

Abbreviations: $A\beta_{42/40}$, Amyloid β $_{42/40}$ ratio; AD, Alzheimer's Disease; APOEε4, Apolipoprotein E type 4 allele; AUC, Area Under the Curve; CERAD WLT recall, Consortium to Establish a Registry for Alzheimer's Disease Word List test Recall trial; CN, cognitive normal; COWAT FAS, Controlled Oral Word Association Test FAS; CSF, cerebrospinal fluid; DDI, Dementia Disease Initiation; HR, Hazard ratio; MCI, mild cognitive impairment; MMSE, Mini Mental Status Evaluation; MRI, magnetic resonance imaging; NfL, Neurofilament Light Chain; NPV, negative predictive value; PPV, positive predictive value; RaC, Recruited as controls; ROC, Receiver operating characteristic; SCD, subjective cognitive decline; SD, standard deviation; TMT-B, Trail Making Test part B; T-tau, Total-Tau; VOSP silhouettes, Visual Object and Space Perception silhouettes.

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https://doi.org/10.1016/j.neurobiolaging.2024.05.016
Received 20 December 2023; Received in revised form 1 May 2024; Accepted 24 May 2024
Available online 29 May 2024

et al., 2013), but positively correlated with increasing CSF t-tau and p-tau (Skillbäck et al., 2013). Increased NfL concentrations are associated with higher age (Vågberg et al., 2015, Khalil et al., 2018), white matter hyperintensities (Bergman et al., 2016, Hjalmarsson et al., 2014), infections in the central nervous system (CNS) (Studahl et al., 2000, Grahn et al., 2013), cognitive decline and brain atrophy (Mattsson et al., 2016). It has been argued that a limiting factor of disease monitoring when using NfL is the lack of normative reference values across age groups (Khalil et al., 2018). Indeed, such values have been published (Simrén et al., 2022), albeit without validation of putative improvements in diagnostic precision. In summary, as evidence point to increases in both CSF t-tau and NfL during aging, there may be diagnostic benefits of using age-adjusted normative reference data to delineate benign age-related increases in CSF t-tau and NfL from increases caused by neuropathology.

Traditional discrete norming procedures are frequently used in medical practice when deriving normative reference groups (Oosterhuis et al., 2016), e.g., age-adjusted cut-offs, and enable interpretations of an individual's results compared to peers in a defined subgroup (e.g. ages 60 through 70). However, this procedure requires large sample sizes, since the cut-offs and test-score distributions are derived from each discrete subgroup, and multiple groups need to be defined to accommodate relevant characteristics (i.e., sex and age) (Henny et al., 2000). This is difficult in practice and produces potentially arbitrary and imprecise reference groups. For example, Sjogren et al. (2001) used age adjusted cut-offs for CSF t-tau based on such reference groups, which can give an unrealistic jump in the cut-off values between age-groups given that the changes in t-tau are presumed to increase smoothly with increasing age. Regression-based norms represent an alternative method where the entire sample is used to model changes in e.g., CSF t-tau or NfL concentrations linearly, or non-linearly with age. Such methods also allow for simultaneous modeling of other pertinent predictors, such as sex (Timmerman et al., 2021), and are therefore more efficient, requiring up to 5.5 times smaller sample sizes than discrete norming (Oosterhuis et al., 2016). Regression-based normative procedures have been implemented in studies of cognition (Lorentzen et al., 2021, Espenes et al., 2020) and MRI morphometry (Potvin et al., 2017, Luo et al., 2015), but have to our knowledge not been validated for fluid biomarkers of neurodegeneration. Cut-offs derived from regression-based norms may improve diagnostics, as it could increase diagnostic accuracy for pathological, rather than healthy age-associated increase in these biomarkers.

We here have developed and assessed age-adjusted regression norms for both CSF t-tau and NfL concentrations based on a sample of cognitively normal (CN, n = 137) participants. To ensure that our CN sample was representative of healthy aging and without signs of preclinical AD, all included participants had normal CSF $A\beta_{42/40}$ ratios at baseline and remained cognitively normal at follow-up (*mean* years = 3.97, *SD* = 1.72). We hypothesize that CSF t-tau and NfL will be positively associated with age in the CN group, and that our age-adjusted norms will provide improved diagnostic accuracy of mild cognitive impairment (MCI) due to AD ($A\beta_{42/40}$ positive cases, AD-MCI) using receiver operating curve (ROC) analyses as compared to unadjusted CSF concentrations. Furthermore, using survival curve analyses, we expect that the age-adjusted norms will better predict progression to future MCI or dementia diagnosis as compared to unadjusted CSF concentrations.

2. Methods

2.1. Study cohort

This study was conducted with data from the Norwegian Dementia Disease Initiation (DDI) cohort. The participants have been recruited since 2013, with follow-ups at approximately two-year intervals. The study is conducted at six locations in Norway: Akershus University Hospital; St. Olav's, University Hospital; Stavanger University Hospital; Haugesund hospital; Betanien hospital; and University Hospital of North Norway. Inclusion criteria were age between 40 and 80 years and native language of Norwegian, Swedish, or Danish. Exclusion criteria were brain trauma or brain disorders like stroke, dementia, severe psychiatric disease, severe somatic disease that might influence cognitive functions or intellectual disability or other developmental disorders. All participants underwent a standard protocol with patient history, and volunteered blood- and CSF samples, brain MRI, neurological and neuropsychological examinations. Recruitments to the study are mainly based on referrals to local memory clinics, in addition to participants responding to advertisements in media/newspapers/news bulletins. Healthy controls are recruited from advertisements in local media, spouses of patients with symptoms of cognitive disorders, and from patients with completed lumbar puncture in connection with orthopaedic surgery that reports no experience of subjective cognitive decline (SCD). Participants experiencing SCD in any cognitive domain, but performing normal on objective neuropsychological tests, are classified according to the SCD-I framework (Jessen et al., 2014). The NIA-AA criteria are used to define MCI (Albert et al., 2011). The MCI group scores lower than expected in one or more cognitive domains but are independent in daily functional ability and do not fulfil the criteria of dementia. Scores 1.5 standard deviations (*SD*) below the normative mean on either the Consortium to Establish a Registry for Alzheimer's Disease Word List task (CERAD-WLT) (Kirsebom et al., 2019), Visual Object and Space Perception (VOSP) silhouettes (Eliassen et al., 2020), Trail Making Test part B (TMT-B) (Espenes et al., 2020) or Controlled Oral Word Association Test (COWAT-FAS) (Lorentzen et al., 2021) are used to define abnormal cognition from normal cognition. See Fladby et al. (2017) for further detailed information regarding clinical assessment and procedures (Fladby et al., 2017).

2.2. CSF biomarker collection, handling, and measurements

All lumbar punctures are sampled before noon. Polypropylene tubes (Thermo Fisher Scientific, MA, USA) are used to collect CSF. The tubes are centrifuged within 4 h at 2000*g* for 10 min at room temperature. The supernatant is transferred to new tubes and frozen directly at −80 °C on site, shipped frozen to a central lab facility at Akershus University Hospital, and kept at −80 °C until analysis. All CSF analyses were performed at either the Department of Interdisciplinary Laboratory Medicine (t-tau assay) or Section of Clinical Molecular Biology (EpiGen) at Akershus University Hospital (NfL and Aβ assays). T-tau was determined using Innotest hTau Ag kit (Fujirebio, Ghent, Belgium). CSF NfL and CSF $A\beta_{1–40}$ and $A\beta_{1–42}$ were measured by the QuickPlex SQ 120 system from Meso Scale Discovery (MSD, MD, USA). NfL was measured in a R-plex format using Human Neurofilament L Assay (K1517XR-2) and $A\beta_{1–40}$ and $A\beta_{1–42}$ in a multiplex setup using V-plex Aβ Peptide Panel 1 (6E10) kit (K15200E-1). The ratio of CSF $A\beta_{1–42}$ to $A\beta_{1–40}$ ($A\beta_{42/40}$ ratio) is used to determine the presence or absence of Aβ plaque pathology. The cut-off (≤.077) for $A\beta_{42/40}$ ratio was determined following using receiver ROC analysis using visual read of [18 F]-Flutemetamol PET scans as the standard of truth (Siafarikas et al., 2021).

2.3. Study design

We included 137 CN participants aged between 41 and 78 years with normal CSF $A\beta_{42/40}$ ratios at baseline that remained CN at latest available follow-up (*M* = 3.97, *SD* = 1.72, range: 1.08 – 8.25 years since baseline). The CN group comprised of 50 cases recruited as controls (RaC) and 87 participants that reported SCD. In addition, we included 144 individuals with MCI and pathological CSF $A\beta_{42/40}$ ratios aged between 50 and 78 years. MCI individuals with Aβ pathology are likely in a prodromal phase of AD (Jack et al., 2017) and were included since clinical impairment should to a certain degree be the result of ongoing neuronal dysfunction and degeneration detectable before pathological neuronal degeneration on MRI (Jack et al., 2011, Dubois et al., 2016). Of

these cases, all had CSF t-tau measurements available. However, NfL was missing for one participant each in the RaC, SCD and MCI groups. Information about APOE- ε4 status was missing in one participant in the SCD group and seven in the MCI group. In the RaC group, one TMT-B, COWAT-FAS and VOSP silhouettes score was missing. For the SCD group one TMT-B and COWAT-FAS score was missing, while five scores were missing for the VOSP silhouettes test. Lastly, for the MCI group, five where missing CERAD-WLT recall and VOSP silhouettes scores and eleven lacked TMT-B scores. To assess the association between CSF t-tau and NfL and progression to future MCI or dementia diagnoses, we also included all cases with age at baseline between 41 and 78 years (matching the age-range of the normative sample) from the DDI cohort with available follow-up data and available baseline CSF t-tau and NfL (n = 429, $M = 4.00$, $SD = 1.91$, range: 0.77 – 9.86 years since baseline).

2.4. Statistical analyses

2.4.1. Between samples comparisons

All statistical analyses were performed in Rstudio, R version 4.3.2 (Team, 2023). Participants in the CN (RaC and SCD) and MCI group included information about age, education, sex, CERAD WLT recall, TMT-B score, COWAT FAS, VOSP silhouettes, CSF t-tau and NfL concentrations, and *APOE-ε4* status. To ensure that the SCD group and RaC groups did not differ significantly in terms of demographics, cognitive performance, and CSF biomarker concentrations, we first performed several between-group comparisons and assessment of between-group differences in age associations to CSF t-tau and NfL. We performed independent sample t-tests for the continuous variables age, t-tau, NfL and the demographically adjusted cognitive tests scores (T-scores with $M = 50$ and $SD = 10$ for CERAD WLT recall, TMT-B score, COWAT-FAS and VOSP silhouettes) with assumed normal distributions. We used Levene's test to assess the homogeneity of variance assumption. When violated ($p<.05$), welch t-test was performed. Chi-squared tests were used to assess between-group differences in sex- and *APOE-ε4* genotype distributions. To further ensure that associations between CSF markers and age were similar between RaC and SCD, we fitted models with age by group interaction terms (here, standardized coefficients are reported). We also performed between-group comparisons between the CN group (RaC + SCD) versus the AD-MCI group (see Table 1).

2.4.2. Developing regression-based norms for t-tau and NfL in a CN sample

The age adjusted regression-based norms for CSF t-tau and NfL were developed following procedures described in previously published literature (Espenes et al., 2020, Kirsebom et al., 2019, Van Der Elst et al., 2006). We used CN individuals with normal CSF $A\beta_{42/40}$ who remained cognitively normal at last available follow-up as the basis for the regression norms. We used linear regression with age as the only predictor. However, a non-linear relationship was also explored by including a squared term (age^2), but only linear associations between age and the biomarkers were observed. To ensure that the assumptions of linear regression were met (e.g., normality of residuals and heteroscedasticity) we assessed Q-Q plots and residuals vs predicted values plots. Due to slight departures from normality in the residual diagnostics, we opted to log-transform both CSF t-tau and NfL prior to final analyses. R^2 effect sizes are reported for each model (see Table 2).

2.4.3. Calculation and evaluation of normative performance

To calculate age-adjusted CSF t-tau and NfL, standardized z-scores for both the CN sample and AD-MCI samples we used the regression norms as previously described. Briefly, predicted CSF t-tau or NfL values from the regression norms were subtracted from observed CSF t-tau or

Table 2
Normative regression models for t-tau and NfL based on CN individuals that remain CN at 2-year follow-up.

Variable	Predictor	b (SE)	*t (p)*	R^2	SD residual
Normative model for CSF t-tau	Intercept	4.635 (0.211)	21.91 **(<.001)**	0.12	0.35
	Age	0.015(0.004)	4.31 **(<.001)**		
Normative model for CSF NfL	Intercept	5.989(0.228)	26.31 **(<.001)**	0.31	0.37
	Age	0.029(0.004)	7.79 **(<.001)**		

The dependent variables were log transformed prior to analyses. b = unstandardized regression coefficient; CN = Cognitive normal; CSF = cerebrospinal fluid; NfL = Neurofilament Light Chain; $p = p$-values; R^2 = explained variance of model; SE = Standard Error; SD = Standard Deviation; t-tau = total-tau; t = t-test statistic.

Table 1
Demographic, cognitive, APOE ε4, t-tau and NfL comparison between RaC vs SCD and total CN (CN + SCD) vs MCI.

	RaC(n = 50)	SCD(n = 87)	t / χ^2 (p)	RaC + SCD(n = 137)	AD-MCI(n = 144)	t / χ^2(p)
Age Mean (SD) [range]	58.56(8.54)[41–77]	60.30(8.53)[41–78]	$t = -1.15$(.253)	59.66(8.54)[41–78]	67.05(7.44)[50–78]	t= - 7.73 **(<.001)**
Education Mean (SD) [range]	14.44(2.60)[10–21]	13.89(2.92)[8–22]	$t = 1.11$(.268)	14.09(2.81)[8–22]	13.35(3.30)[4–22]	$t = 2.02$**(<.05)**
Female n (%)	32(64.00)	40 (45.98)	$\chi^2 = 3.45$(.063)	72(52.55)	73(50.69)	χ^2 =.04(.847)
CERAD recall Mean (SD)	53.18(8.58)	50.21(8.74)	$t = 1.93$(.056)	51.29(8.77)	32.40(9.86)	$t = 16.68$ **(<.001)**
TMT-B score Mean(SD)	52.20(9.27)	50.17(9.06)	$t = 1.24$(.217)	50.91(9.16)	35.24(13.07)	$t = 11.35$* **(<.001)**
COWAT-FAS Mean (SD)	53.80(10.33)	50.94(8.66)	$t = 1.72$(.089)	51.98(9.36)	43.32(11.32)	$t = 6.98$* **(<.001)**
VOSP silhouettes Mean (SD)	55.22(9.15)	53.45(9.81)	$t = 1.03$(.307)	54.11(9.57)	44.52(10.89)	$t = 7.67$**(<.001)**
APOE ε4 status (%)	24(48.00)	26(30.23)	$\chi^2 = 3.56$(.06)	50(36.76)	104(75.91)	$\chi^2 = 40.96$ **(<.001)**
t-tau Mean (SD)	280.26(96.02)	265.85(89.87)	$t = 0.88$(.380)	271.11(92.08)	620.00(293.10)	$t = -13.60$* **(<.001)**
NfL Mean (SD)	2561.76(1557.38)	2565.89(1203.03)	$t = -0.02$ (.986)	2564.391336.73	4528.40(3432.90)	$t = -6.35$* **(<.001)**

AD-MCI = Mild Cognitive Impairment due to Alzheimer's Disease; APOEε4 = Apolipoprotein E type 4 allele; CERAD WLT = Consortium to Establish a Registry for Alzheimer's Disease Word List Test; CN = cognitive normal; COWAT-FAS = Controlled Oral Word Association Test FAS; NfL = neurofilament light chain; $p = p$-values; RaC = Recruited as Controls; SCD = Subjective Cognitive Decline; SD = Standard Deviation; t = t-test statistic; t-tau = total tau; TMT-B score = Trail Making Test part B; VOSP = Visual Object and Space Perception Battery; χ^2 = Chi-squared test; * = Welch t-test due to non-equal variances between groups.

NfL values in the samples and divided by the SD of the residuals from the regression normative model. For example: [z-score = (log(observed biomarker level)) – log(predicted biomarker level)/(residual *SD*)].

2.4.4. Receiver operating characteristic (ROC) curve analyses

We then compared unadjusted raw values and the age adjusted regression norms with the CN group as controls, and AD-MCI as the standard of truth in ROC analyses to evaluate diagnostic accuracy for CSF t-tau and NfL. An area under the curve (AUC) of 0.7–0.8 is considered acceptable, while 0.8–0.9 is excellent (Mandrekar, 2010). Delongś test (for paired AUCs) was used to assess differences between the AUCs (see Table 3), and the Youden index was used to calculate the optimum cut-off for the models. Negative Predictive Values (NPVs) and Positive Predictive Values (PPVs) were computed for all models.

2.4.5. Assessment of age associations with CSF markers in the AD-MCI group

To better understand how the norms may influence resulting AUCs, we also include an assessment of the associations (Pearsonś R correlation) between age and the CSF biomarkers in the AD-MCI group.

2.4.6. Survival curve analyses

We first applied the aforementioned cut-offs for both age-adjusted and raw values of CSF t-tau and NfL in all participants in the DDI cohort who had CSF markers at baseline, and available follow-up assessments. We then fitted Cox Proportional Hazard models using both age-adjusted and non-adjusted cut-offs to predict future progression to MCI or dementia. Hazard Ratios (HR) and their associated 95 % confidence intervals for pathological CSF markers as compared to non-pathological CSF markers are reported.

2.5. Ethics

The study has been approved by the Regional Committees for Medical and Health Research Ethics in Norway (2013/150/REK sør-øst) and was conducted in line with the guidelines provided by the Helsinki declaration of 1964, 2013 revision, and the Norwegian Health and Research act. All participants gave a written informed consent before participating in the study.

3. Results

3.1. Between-group comparisons between the RaC and SCD participants within the CN group

There were no statistical differences between the RaC and SCD participants for neither age, CSF t-tau or NfL concentrations, cognitive performance, sex distribution nor *APOE-ε4* status. See Table 1 for details. We also found that there were no significant differences in age association (linear regression with age*group interaction) with neither CSF t-tau (p =.645, Fig. 1A) nor NfL (p =.203, Fig. 1B) between the RaC and SCD participants. Moreover, although the included RaC and SCD groups were deemed cognitively normal at baseline and latest follow-up, we also recently demonstrated that there are no significant differences in linear cognitive performance over time between these groups (Hemminghyth et al., 2024). Taken together, this suggests that these groups can be combined as a CN group for regression norming.

3.2. Between-group comparisons between the CN and AD-MCI

The AD-MCI group was older, had lower education, performed worse on all cognitive measures, had higher CSF t-tau and NfL concentrations and had more participants with APOE-ε4 genotypes as compared to the CN group. Sex distributions were similar between the groups. See Table 1 for details.

3.3. Age association with CSF t-tau and NfL in CN cases

In the normative CN sample, both CSF t-tau (b = 0.015, p<.001, Fig. 2A) and CSF NfL concentrations (b = 0.029, p<.001, Fig. 2B) increased with age. However, whilst age explained 12 % of the variance in t-tau concentrations, age explained 31 % in the NfL concentrations, suggesting a stronger age-related increase in NfL than for t-tau. See Table 2 for details. These regression norms were subsequently computed for both our CN and AD-MCI sample as described in the methods section.

3.4. ROC, PPV and NPV analysis for t-tau and NfL

Contrary to our hypothesis, our results showed that the model with age-adjusted CSF t-tau norms was significantly worse (AUC =.861) compared to the unadjusted CSF t-tau model (AUC =.900, p<.001) (Table 3, Fig. 3A). Similar results were also shown for CSF NfL, where the age-adjusted norms produced significantly lower AUC (.727) as compared to unadjusted model (AUC =.810, p<.001) (Table 3, Fig. 3B).

Additionally, the age-adjusted models had reduced efficacy in accurately identifying positive cases, reflected in lower PPVs for t-tau (PPV = 0.824) and NfL (PPV = 0.675) compared to the unadjusted models (t-tau: PPV = 0.907 and NfL: PPV = 0.747). Furthermore, the age-adjusted models also had decreased accuracy in distinguishing healthy individuals indicated by lower NPVs for both t-tau and NfL (t-tau: NPV = 0.760 and NfL: NPV = 0.685) compared to unadjusted models for t-tau (NPV = 0.773) and NfL (NPV = 0.774). See Table 3.

3.5. Age association with CSF t-tau and NfL in AD-MCI cases

To elucidate the reductions in AUCs when using our regression norms, we performed correlations between age and the CSF biomarkers, both for raw-values and the age-adjusted norms in the AD-MCI group. Here, CSF NfL correlated with age (r = 0.37, p<.001, Fig. 4C), but explained less variance in this group (13.7 %) than in the normative CN sample (31 %, see above). This resulted in a slight overadjustment of expected age-effects using normative CSF NfL values resulting in a negative association to age in this group (r = -0.19, p = .023, Fig. 4D). CSF t-tau concentrations did not increase with age (r = -0.01, p = .938,

Table 3
ROC analyses for CSF t-tau and CSF NfL. Controls are CN and SCD with normal biomarkers for Aβ, while cases are MCI with pathological biomarkers for Aβ.

	AUC	95 % CI	PPV	NPV	CN Aβ- / MCI Aβ+	Optimal cut-off	Specificity	Sensitivity	DeLong's test
Unadjusted t-tau	0.900	0.865–0.935	0.907	0.773	137/144	>398*	0.920	0.743	Reference
Age adjusted regression norm for t-tau	0.861	0.818–0.903	0.824	0.760		>0.861†	0.832	0.750	D = -4.48 p<.001
Unadjusted NfL	0.810	0.759–0.861	0.747	0.774	135/143	>2898*	0.711	0.804	Reference
Age adjusted regression norm for NfL	0.727	0.668–0.786	0.675	0.685		>0.280†	0.630	0.727	D = - 4.59 p<.001

AUC = Area Under the Curve; Aβ+/- = Amyloid plaque pathology positive or negative; CI = Confidence Interval; CN = cognitive normal, CSF = cerebrospinal fluid; D = DeLong's test; MCI = mild cognitive impairment; NfL = neurofilament light chain; NPV = negative predictive value; PPV = positive predictive value; p = p-values; ROC = receiver operating characteristic curve analysis; t-tau = total-tau; SCD = subjective cognitive decline. * Cut-off in pg/mL; † Cut-off in z-score.

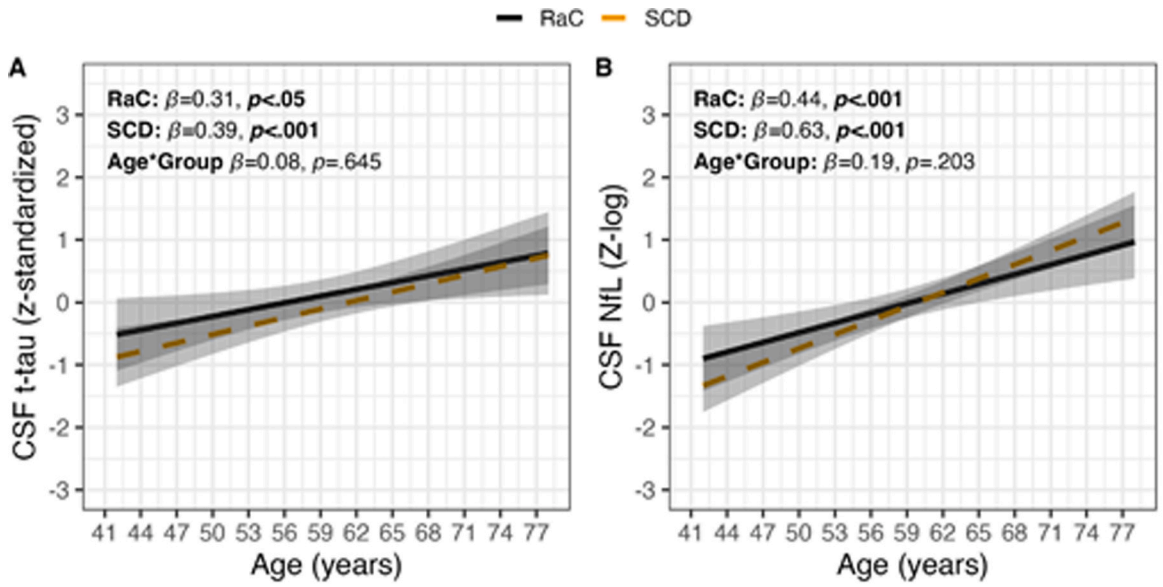

Fig. 1. Illustrates the analyses performed to ensure comparability of the recruited as controls (RaC) and subjective cognitive decline (SCD) in the cognitive normal (CN) group. Associations between age and cerebrospinal fluid (CSF) t-tau (**A**) and NfL (**B**) in the RaC (black solid lines) and SCD groups (yellow dashed lines). Here, CSF markers (y-axes) are shown as standardized log transformed values (Z-log) and pertinent statistical results are reported using standardized coefficients.

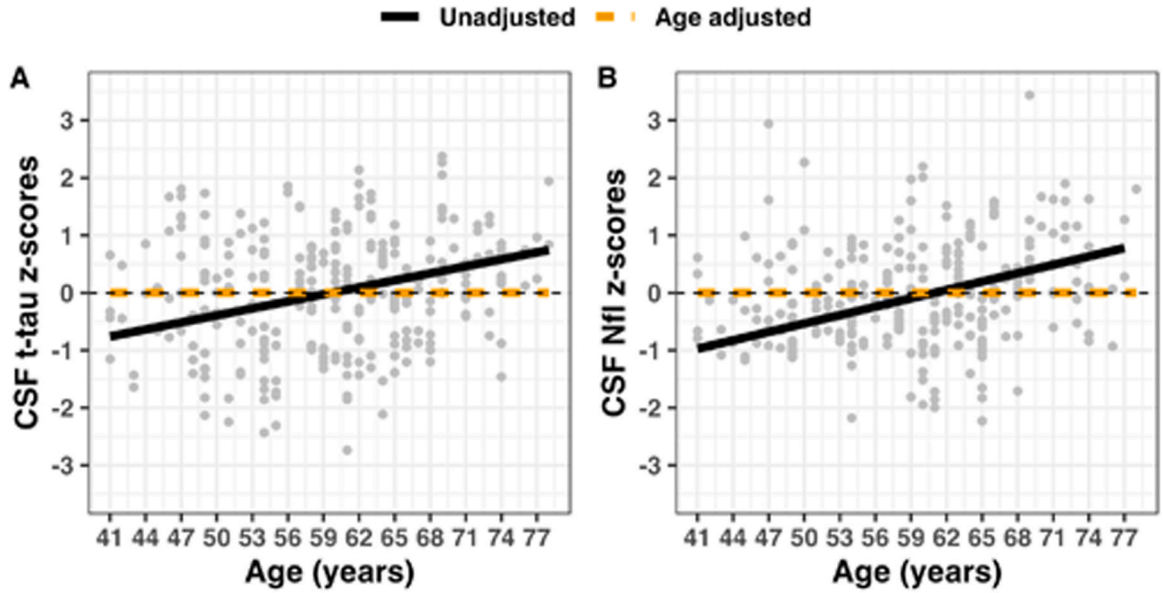

Fig. 2. Illustration of normative age association to biomarkers in the normative sample. (**A**) Shows CSF t-tau, (**B**) shows CSF NfL. The dashed yellow line represents our norms (the reference level) laying approximately around z = 0, while the regression line (solid black line) shows the unadjusted CSF biomarker concentrations (unadjusted z-scores), increasing with age linearly for both CSF t-tau and CSF NfL.

Fig. 4A). As with NfL, a corresponding slight overadjustment of age using normative CSF t-tau values was observed (r = -0.17, p = .067, Fig. 4B).

3.6. Survival curve analyses of participants progressing to MCI or dementia

Table 4 provides an overview of the demographic characteristics related to the progression to MCI or dementia. Out of the total participants (n = 429), 292 were stable in either the CN (n = 183) or the MCI (n = 107) group, whereas 82 participants experienced progression to either MCI (n = 44) or dementia (n = 38). Notably, 56 participants transitioned from MCI to CN. This transition underscores the fact that MCI diagnostic criteria rely on the presence of only one score lower than expected in a cognitive domain, which is recognized for occasionally yielding false positive MCI diagnoses (Jak et al., 2016). In Fig. 5 we present the hazard ratios (HR) of age-adjusted CSF t-tau (5A), unadjusted CSF t-tau (5B), age-adjusted CSF NfL (5 C) and unadjusted CSF NfL (5D) in participants progressing to MCI or dementia. Notably, both age-adjusted CSF t-tau (HR = 2.29, 95 % CI [1.47, 3.55], p<.01) and unadjusted CSF t-tau (HR = 3.39, 95 % CI [2.19, 5.26], p<.001) positive groups were significantly associated with clinical progression when compared to their corresponding CSF t-tau negative counterparts. Similar results were seen for age-adjusted CSF NfL (HR = 1.89, 95 % CI [1.20, 2.98], p<.01) and unadjusted CSF NfL (HR = 3.17, 95 % CI [1.99, 5.04], p<.001) positive groups. Overall, a slight advantage was observed for models with

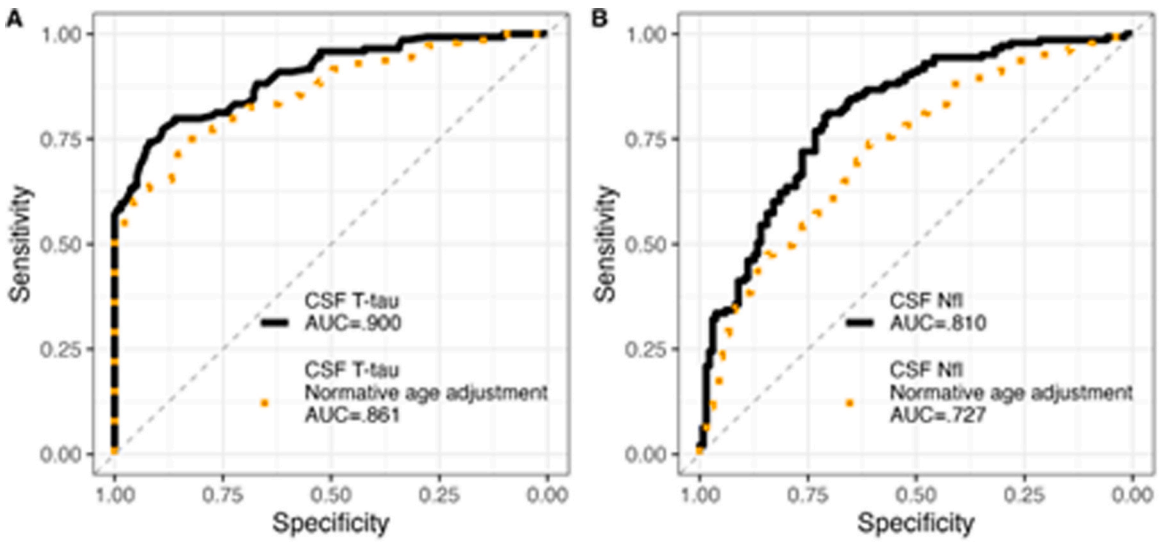

Fig. 3. Comparison of diagnostic performance for mild cognitive impairment with Aβ+ (AD-MCI) as compared to cognitively normal (CN) persons (recruited as controls and subjective cognitive decline) with normal Aβ biomarkers (CN Aβ-) in cerebrospinal fluid. Area under the curve (AUC) are shown for (**A**) unadjusted CSF t-tau (solid black line) and age adjusted regression norms for CSF t-tau (dashed yellow line). (**B**) Unadjusted CSF NfL (solid black line) and age adjusted regression norms for CSF NfL (dashed yellow line). The dashed grey line corresponds to random chance (AUC = 0.5).

unadjusted biomarker cut-offs.

4. Discussion

We here have developed age-adjusted regression norms for CSF t-tau and NfL based on a CSF determined Aβ negative sample of cognitively normal participants, that also remained cognitively normal over time. We demonstrated that while both CSF t-tau and NfL concentrations linearly increased with higher age in the CN group, NfL showed markedly stronger associations with age than t-tau. However, contrary to our hypotheses, our age-adjusted norms for CSF t-tau and NfL did not improve diagnostic accuracy for MCI with pathological CSF $A\beta_{42/40}$ ratios than models with unadjusted CSF concentrations. Moreover, unadjusted CSF t-tau and unadjusted CSF NfL had higher hazard ratios for progressing to future MCI and dementia diagnoses compared to the age-adjusted norms.

A common feature of normative reference data is to assess whether there is a deviation from an expected mean in an ageing population. For many cognitive tests, such as episodic memory performance, elderly individuals are expected to remember less than their younger counterparts (Espenes et al., 2022). Consequently, an elderly person is allowed to perform worse than a younger person before being diagnosed with cognitive impairment. In our study, the same logic applies, where an elderly person must have markedly higher CSF t-tau and NfL concentrations than a younger person to be classified as pathological. Thus, a possible explanation why age-adjusted norms did not improve diagnostic accuracy in our study could be that older persons with MCI are expected to have very high concentrations of CSF t-tau and NfL markers (above the expected mean for this age group), and many may thus be incorrectly classified as non-pathological. This discrepancy may arise because CSF t-tau levels did not exhibit an age-related increase in the AD-MCI groups, as presumed by age-adjusted norms, resulting in an unintended overcompensation and higher rate of false negatives. Conversely, a younger individual with AD may only require a moderate increase in CSF t-tau and NfL markers to be classified as pathological. Moreover, since CSF NfL showed the most marked age-related increase in our normative sample (31 % explained variance) and while significant, to a lesser degree in the AD-MCI group (13.7 % explained variance), leading to a significant overestimation of age using the normative data resulting in an even greater loss in diagnostic accuracy for this marker.

Studies by Mattsson et al. (2009) and Lleó et al. (2019) have both reported a positive association between age and t-tau levels in healthy controls. However, in individuals with AD dementia, these associations were either non-significant or negative. Both studies also observed positive correlations with age in cases of MCI but did not control for amyloid status in their analyses. This oversight may explain the non-significant age association observed in our AD-MCI group. The consistent elevation of t-tau in the AD continuum, as noted by Zetterberg et al. (2007), suggests that t-tau levels might be increased due to disease-associated neurodegeneration throughout the course of the disease, independent of age. Thus, a negative association with age found in e.g. the Lleó et al. (2019) AD dementia group, could be due to younger cases with likely earlier onset AD dementia having higher t-tau associated with faster disease progression (Wallin et al., 2010, Blom et al., 2009, Kaur et al., 2020). T-tau elevation in our AD-MCI cases putatively associated with cortical neurodegeneration (Holper et al., 2022), may similarly diminish or completely negate the age association typically seen in cognitively healthy controls.

Numerous studies have tried to distinguish between healthy and pathological brain aging, but a clear consensus is still lacking. However, previous evidence has shown that Aβ plaques and neurofibrillary tangles are present in cognitively normal aging (Saha and Sen, 2019). AD has a long prodromal period without clinically evident impairment. These cases could be at increased risk of developing cognitive impairment over time, implying that this is not a part of healthy aging (Kloske et al., 2021, Harada et al., 2013, Draganski et al., 2013). Our results indicate that age-adjusted regression norms do not improve the diagnostic accuracy of cognitive impairment in cases with CSF $A\beta_{42/40}$ pathology, nor does it improve diagnostic performance in predicting progression to MCI or dementia. This could substantiate the fact that age-associated increases in CSF t-tau and NfL may not be due to healthy aging, but rather that the participants may not have developed cognitive impairment yet. However, neurobiological changes are common with aging (Lee and Kim, 2022), and the prevalence of dementia in the studied age-range is comparatively low (Gjøra et al., 2021). The increase in the CSF markers with normal aging may reflect incipient neurodegenerative or cerebrovascular changes increasing with higher age, and also associated with signs of brain atrophy and higher concentrations of CSF t-tau and NfL (Vågberg et al., 2015).

Other studies have reported stronger associations between age and t-tau concentrations (Sjogren et al., (2001) $r = 0.60$, $p<.001$) as well as

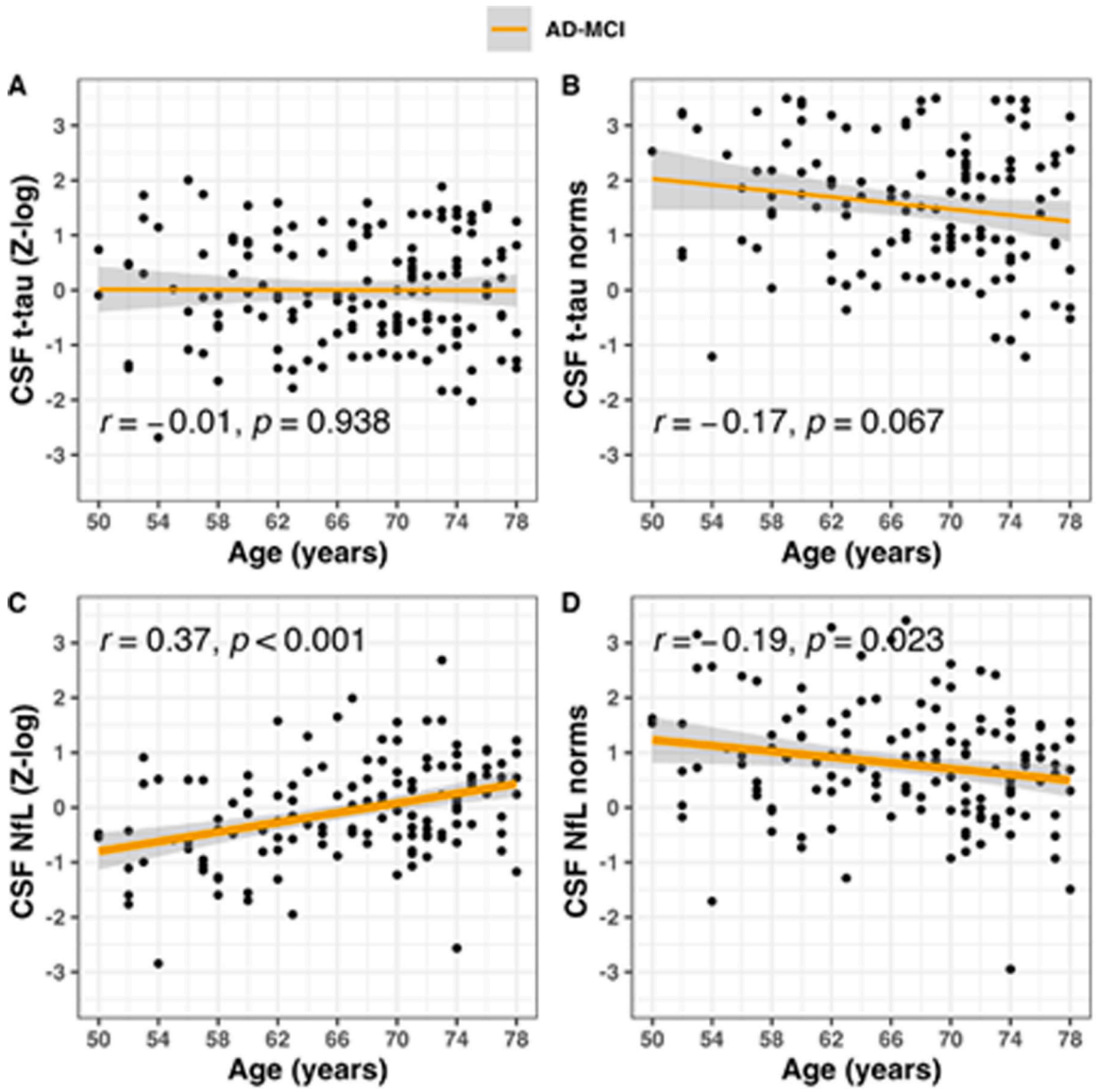

Fig. 4. Illustration of normative age association to biomarkers in the AD-MCI sample. (**A**) Shows unadjusted CSF t-tau, (**B**) shows age adjusted CSF t-tau, **(C)** shows unadjusted CSF NfL and **(D)** shows age adjusted CSF NfL. The solid yellow line represents our norms (the reference level), while the solid black circles display the unadjusted CSF biomarker concentrations. CSF markers (y-axes) are shown as standardized log transformed values (Z-log).

Table 4
Demographics of participant progression to MCI or dementia.

	Follow-up time ***N***Mean years(SD) [Range]	**Age at baseline** Mean age(SD) [Range]	**Sex** Female n (%)
All	***429***4.00(1.91)[0.769 – 9.86]	62.8 (8.79)[41– 78]	235(54.8 %)
Stable CN	***183***4.36(1.84)[1.57 – 9.67]	61.7 (8.88)[41– 78]	99(54.1 %)
CN to dementia	***1***1.97(-)[1.97 – 1.97]	77.0 (-)[77 – 77]	0(0 %)
CN to MCI	***44***3.55(1.54)[1.93, 6.74]	64.6 (8.50)[41 – 78]	28(63.6 %)
MCI to CN	***56***4.71(2.07)[1.30 – 9.36]	60.0 (8.56)[40 – 74]	30(53.6 %)
MCI to dementia	***38***2.61(1.38)[0.769 – 6.19]	67.5 (7.0)[53 – 78]	19(50.0 %)
Stable MCI	***107***3.69(1.93)[1.05 – 9.86]	63.3 (8.66)[41 – 77]	59(55.1 %)

CN = cognitive normal; MCI = mild cognitive impairment; SD = Standard deviation.

age and NfL concentrations (Vågberg et al. (2015) $R^2 = 0.649$, p<.001) compared to our findings. However, we speculate that the comparatively weaker associations with age in our normative sample may be due to our strict definition of cognitive normalcy, both at baseline and over time. In addition, we also controlled for the possibility of inadvertently including participants with prodromal AD by controlling for CSF determined amyloid pathology. Thus, our norms may be less strict than when developing normative reference data from healthy participants without imposing the same rigor. Moreover, if the findings and conclusions from our study are confirmed by future studies, the use of norms with even higher expected normative age increase could potentially lead to even worse diagnostic accuracies, and prognostic performance. Moreover, although a non-linear increase with age has been seen for serum NfL in participants >60 years of age (Simrén et al., 2022, Khalil et al., 2020), both studies were performed with presumed healthy participants, but did not include information about amyloid status in all participants. Inclusion of wider age-ranges and influence of peripheral sources affecting NfL (Barro et al., 2020, Pichet Binette et al., 2023) may also contribute to different findings concerning a non-linear increase with age, particularly in the eldest age groups.

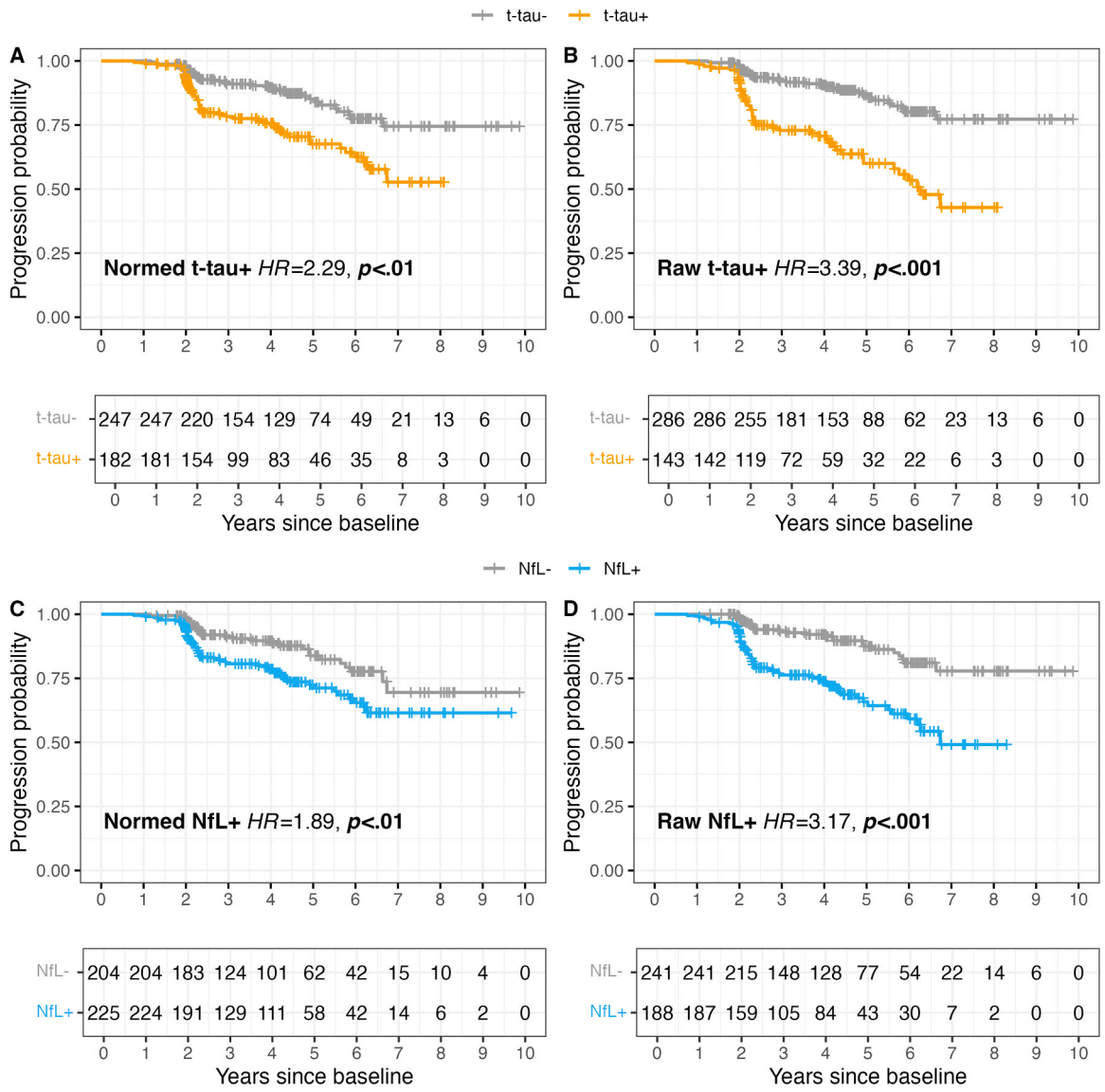

Fig. 5. Illustration of survival curve analyses of participants developing mild cognitive impairment (MCI) or dementia. Panel (**A**) presents normed CSF t-tau positive (solid yellow line) against normed CSF t-tau negative (solid gray line) participants, (**B**) unadjusted CSF t-tau positive against unadjusted CSF t-tau negative, (**C**) normed CSF NfL positive (solid blue line) against normed CSF NfL negative (solid grey line) and (**D**) unadjusted CSF NfL positive against unadjusted CSF NfL negative. For each of the comparisons hazard ratios (HR) and p-values are provided. Additionally, the figure provides an overview of the participant count at each year follow-up for both CSF t-tau and CSF NfL positive and negative, together with age adjusted and unadjusted groups.

There may be a continuum between more benign age-related brain changes and their associated CSF markers, and those associated with progressive neurodegenerative conditions, such as AD. We would also expect a certain degree of variation in neurodegenerative processes between individuals in the same age-group. Therefore, some non-symptomatic elderly with increased CSF biomarkers of neurodegeneration could reflect a pathological change that over time could lead to cognitive impairment (Thal et al., 2004). As previously shown, our CN group remained cognitively normal through the observation period, and even showed improved cognition over time (Hemminghyth et al., 2024), putatively due to practice effects (Bartels et al., 2010). Thus, we hypothesize that due to the overlap between normal aging and pathology with regards to CSF t-tau and NfL, the use of age-adjusted norms may obscure a significant increase in these CSF markers associated with incipient AD-related mechanisms, which may in part be independent of the age-related increase seen in healthy adults. This interpretation is supported by the lack of age-related increase in CSF t-tau concentrations, and lesser age associated increase for CSF NfL concentrations in the AD-MCI group. Putatively pointing to disease driven, rather than age driven increase in these markers.

We generally found lower AUCs for CSF NfL compared to CSF t-tau AUCs, which likely correspond to reports noting that CSF NfL is a less specific biomarker for AD as compared to CSF t-tau (Mattsson et al., 2016, Khalil et al., 2018) and as mentioned, that the age associated increase seems more prominent for CSF NfL than for CSF t-tau. CSF NfL is not specifically associated with Aβ pathology but with cognitive decline and brain atrophy in general (Mattsson et al., 2016), which may explain why CSF NfL concentrations also increase with ageing in the AD-MCI group. As a marker of axonal damage, increased CSF NfL might therefore represent white matter tissue loss (Vågberg et al., 2015). CSF t-tau is associated with grey matter degeneration (Wang et al., 2012) and is highly expressed in unmyelinated axons of the cortex (Zetterberg, 2017). A reduction in both grey and white matter is associated with aging (Drayer, 1988), and supports our results that these CSF biomarkers increases with age, putatively reflecting neurodegenerative processes that nevertheless have not caused clinical cognitive impairment.

While age is considered one of the greatest risk factors for AD (Saha and Sen, 2019). Aging is also associated with decline in multiple cognitive domains like memory, executive functions, and information processing speed (Alexander et al., 2012) appearing in parallel to reduced performance of other organ systems with acceleration after 50 years of age (Mendonca et al., 2017). However, when using more rigorous criteria for defining healthy participants, findings suggest that the effect of age on cognition might be over-estimated (Harrington et al., 2017, Borland et al., 2020). While the influence of age has been found to be small or nonexistent in simpler tasks, age-effects may be larger in tasks with more complex cognitive demands (Harrington et al., 2017). This appears consistent with the notion that healthy aging does entail some changes that make our brains less efficient, but to a lesser extent than commonly believed. This is also supported by the prominent practice effects on cognitive tests demonstrated in the CN group (Hemminghyth et al., 2024).

While we focused on CSF, the use of blood-based biomarkers is more available and less invasive, making it a promising choice for first line screening in the future. Plasma and CSF NfL correlate strongly (Hansson et al., 2017, Gisslén et al., 2016), and also show an association with age (Simrén et al., 2022). However, plasma NfL is influenced by both the central- and peripheral nervous system (Barro et al., 2020), as well as body-mass-index and kidney function (Pichet Binette et al., 2023). And while normative reference data has been published (Simrén et al., 2022), to our knowledge, diagnostic and prognostic value of using such norms for plasma markers have not yet been assessed. While measurements of CNS t-tau in blood has been hampered by peripheral sources, and do not correlate with CSF t-tau, a new assay that targets CNS specific tau-forms (Brain-Derived tau) have recently been developed for serum and plasma (Gonzalez-Ortiz et al., 2023), and shown promise as a blood-based marker for CSF t-tau associated neurodegeneration in AD (Gonzalez-Ortiz et al., 2024).

A limitation of our study is that we did not include the brain MRI data, nor information about other potential underlying pathologies that may cause neurodegeneration, such as vascular disease. However, all controls and cases (AD-MCI) were thoroughly screened and adhered to the inclusion and exclusion criteria, securing that clinical or biological markers of disease should not be due to severe somatic illness. This is also ensured by only including a group of CN that remained demonstrably CN over time. We also included both CN participants recruited as controls, not reporting SCD and those reporting SCD in our regression-norms. While individuals with SCD have increased risk of future MCI and dementia (Slot et al., 2019, Mitchell et al., 2014), the majority of those with SCD will not progress to cognitive impairment (Jessen et al., 2010, Eckerstrom et al., 2016), and the experience of SCD may be unspecific to neurodegenerative disease, and perhaps in most cases likely a part of normal aging (Hessen et al., 2017). This assumption is also supported by results showing that RaC and SCD samples did not significantly differ with regards to CSF t-tau or NfL concentrations, their association with age, or their results on cognitive tests at baseline, nor over time (Hemminghyth et al., 2024). Considering the common practice of establishing cognitive norms based on presumed healthy individuals without accounting for neurodegenerative diseases or examination of CSF biomarkers, it is a noteworthy strength in our study that we possess a cohort of CN participants based on cognitive assessments and absence of pathology in CSF (Aβ, t-tau and NfL) longitudinally.

Another limitation of this study is the use of cut-offs based on participants with MCI and Aβ pathology in CSF, rather than employing general cut-offs for neurodegeneration based on CSF t-tau and NfL. However, our study is primarily focused on AD, and the utilization of CSF $A\beta_{42/40}$ pathology is specific for AD (Tapiola et al., 2009), occurring before tau-related neurodegeneration (Jack et al., 2017). This approach is therefore considered sufficient for identifying the presence of prodromal AD, which constitutes the majority of dementia cases (Winblad et al., 2016). Yet, it should be noted that these cut-offs are not generalizable to other conditions that leads to dementia, such as vascular dementia or Lewy body dementia.

5. Conclusion

Age-adjusted regression norms for CSF t-tau and NfL did not enhance diagnostic accuracy of clinical impairment due to CSF $A\beta_{42/40}$ pathology in our study. Furthermore, our age-adjusted norms did not improve diagnostic performance in predicting the prognosis to MCI or dementia within our cohort. One possible explanation is that normative reference data might obscure significant increase in these CSF markers caused by AD, irrespective of age.

CRediT authorship contribution statement

Tormod Fladby: Writing – review & editing, Project administration, Investigation, Funding acquisition. **Knut Waterloo:** Writing – review & editing, Investigation. **Berglind Gísladóttir:** Writing – review & editing, Investigation. **Jacob Espenes:** Writing – review & editing, Investigation. **Lene Pålhaugen:** Writing – review & editing, Investigation. **Per Selnes:** Writing – review & editing, Investigation. **Ingvild Vøllo Eliassen:** Writing – review & editing, Investigation. **Jonas Jarholm:** Writing – review & editing, Investigation. **Gøril Rolfseng Grøntvedt:** Writing – review & editing, Investigation. **Kaja Nordengen:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Bjørn-Eivind Kirsebom:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Stephanie Lindgård Knudtzon:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

Acknowledgements

The project was funded by the Norwegian Research Council, JPND/PMI-AD (NRC 311993) and from the Norwegian Regional Health Authorities (Helse Sør-Øst (25598) and Helse-Nord (HNF1540-20; HNF1569-21)).

Competing interests

BEK has served as a consultant for Biogen and Eisai. TF has served as a consultant and at the advisory boards for Biogen, Eisai, Novo Nordisk, Eli Lilly and Roche. PS has served as a consultant for Roche. SK, KN, GRG, JJ, BG, IE, LP, JE and KW have no disclosures.

Verification

All authors have contributed to the work according to the Vancouver convention and agree with the presented findings. This paper is based on original data, has not been published before nor is being considered for publication in another journal.

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