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Plasma tau, neurofilament light chain and amyloid-β levels and risk of dementia; a population-based cohort study

Frank de Wolf,[1,2,*] Mohsen Ghanbari,[3,4,*] Silvan Licher,[3] Kevin McRae-McKee,[1] Luuk Gras,[2] Gerrit Jan Weverling,[2] Paulien Wermeling,[2] Sanaz Sedaghat,[5] M. Kamran Ikram,[3,6] Reem Waziry,[7] Wouter Koudstaal,[2,8] Jaco Klap,[2] Stefan Kostense,[2] Albert Hofman,[7] Roy Anderson,[1] Jaap Goudsmit[7,9,10] and M. Arfan Ikram[3,7]

*These authors contributed equally to this work.

CSF biomarkers, including total-tau, neurofilament light chain (NfL) and amyloid-β, are increasingly being used to define and stage Alzheimer's disease. These biomarkers can be measured more quickly and less invasively in plasma and may provide important information for early diagnosis of Alzheimer's disease. We used stored plasma samples and clinical data obtained from 4444 non-demented participants in the Rotterdam study at baseline (between 2002 and 2005) and during follow-up until January 2016. Plasma concentrations of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} were measured using the Simoa NF-light® and N3PA assays. Associations between biomarker plasma levels and incident all-cause and Alzheimer's disease dementia during follow-up were assessed using Cox proportional-hazard regression models adjusted for age, sex, education, cardiovascular risk factors and *APOE* ε4 status. Moreover, biomarker plasma levels and rates of change over time of participants who developed Alzheimer's disease dementia during follow-up were compared with age and sex-matched dementia-free control subjects. During up to 14 years follow-up, 549 participants developed dementia, including 374 cases with Alzheimer's disease dementia. A $\log_2$ higher baseline amyloid-β_{42} plasma level was associated with a lower risk of developing all-cause or Alzheimer's disease dementia, adjusted hazard ratio (HR) 0.61 [95% confidence interval (CI), 0.47–0.78; $P < 0.0001$] and 0.59 (95% CI, 0.43–0.79; $P = 0.0006$), respectively. Conversely, a $\log_2$ higher baseline plasma NfL level was associated with a higher risk of all-cause dementia [adjusted HR 1.59 (95% CI, 1.38–1.83); $P < 0.0001$] or Alzheimer's disease [adjusted HR 1.50 (95% CI, 1.26–1.78); $P < 0.0001$]. Combining the lowest quartile group of amyloid-β_{42} with the highest of NfL resulted in a stronger association with all-cause dementia [adjusted HR 9.5 (95% CI, 2.3–40.4); $P < 0.002$] and with Alzheimer's disease [adjusted HR 15.7 (95% CI, 2.1–117.4); $P < 0.0001$], compared to the highest quartile group of amyloid-β_{42} and lowest of NfL. Total-tau and amyloid-β_{40} levels were not associated with all-cause or Alzheimer's disease dementia risk. Trajectory analyses of biomarkers revealed that mean NfL plasma levels increased 3.4 times faster in participants who developed Alzheimer's disease compared to those who remained dementia-free ($P < 0.0001$), plasma values for cases diverged from controls 9.6 years before Alzheimer's disease diagnosis. Amyloid-β_{42} levels began to decrease in Alzheimer's disease cases a few years before diagnosis, although the decline did not reach significance compared to dementia-free participants. In conclusion, our study shows that low amyloid-β_{42} and high NfL plasma levels are each independently and in combination strongly associated with risk of all-cause and Alzheimer's disease dementia. These data indicate that plasma NfL and amyloid-β_{42} levels can be used to assess the risk of developing dementia in a non-demented population. Plasma NfL levels, although not specific, may also be useful in monitoring progression of Alzheimer's disease dementia.

1 Department of Infectious Disease Epidemiology, School of Public Health, Imperial College London, UK
2 World Without Disease Accelerator, Data Science and Prevention Biomarkers, Johnson and Johnson, Leiden, The Netherlands
3 Department of Epidemiology, Erasmus MC-University Medical Center Rotterdam, Rotterdam, The Netherlands

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4 Department of Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5 Department of Preventive Medicine, Northwestern University, Feinberg School of Medicine, Chicago, Illinois, USA
6 Department of Neurology, Erasmus MC-University Medical Center Rotterdam, Rotterdam, The Netherlands
7 Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA
8 Lucidity Biomedical Consulting, Calle Emir 11, Granada, Spain
9 Amsterdam Neuroscience, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
10 Department of Immunology and infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, USA

Correspondence to: Frank de Wolf
Department of Infectious Disease Epidemiology
School of Public Health, Imperial College London
St Mary's Campus, Norfolk Place, London W2 1PG, UK
E-mail: f.dewolf@imperial.ac.uk

Correspondence may also be addressed to: Mohsen Ghanbari
Department of Epidemiology
Erasmus University Medical Center Rotterdam
Wytemaweg 80, 3015 CN Rotterdam, The Netherlands
E-mail: m.ghanbari@erasmusmc.nl

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Abbreviations: MCI = mild cognitive impairment; NfL = neurofilament light chain

Introduction

Dementia is the most common neurodegenerative disease of the elderly, characterized by behavioural changes and the loss of cognitive and social functioning (Goedert, 2015; Alzheimer's Association, 2016). It is most frequently diagnosed as the clinical outcome of Alzheimer's disease (Burns and Iliffe, 2009). Alzheimer's disease is defined by its underlying pathological process of amyloid-β plaques and neurofibrillary tau tangle formation in the brain, accompanied by progressive neuronal and synaptic loss (Braak *et al.*, 2006; Serrano-Pozo *et al.*, 2011; Nisbet *et al.*, 2015). A preclinical phase of perhaps 20 years or more may occur before the clinical diagnosis of Alzheimer's disease dementia, in which no or only subtle symptoms of disease are seen (Villemagne *et al.*, 2013; Goudsmit, 2016; Verlinden *et al.*, 2016). This gives the opportunity to postpone the onset of disease or even prevent Alzheimer's disease dementia by intervening on the preclinical phase of disease. Such early intervention requires timely identification of individuals at high risk of developing Alzheimer's disease by using quantitative biomarkers that are preferably easily accessible and minimally invasive.

Quantitative measurement of proteins that are associated with the neuropathological characteristics of Alzheimer's disease, including amyloid-β deposition, tau-tangle formation and neurodegeneration, in CSF have been suggested as part of a biological definition of Alzheimer's disease (Jack *et al.*, 2018). In this context, CSF levels of amyloid-β_{42}, tau and, more recently, neurofilament light chain (NfL) proteins have been associated with cognition and Alzheimer's disease (Mattsson *et al.*, 2016*a*, 2017; Zetterberg *et al.*, 2016; Olsson *et al.*, 2019). Studies in smaller populations have also revealed that the CSF levels of these proteins, each individually and in combination, are associated with hippocampal volume (Hilal *et al.*, 2018) and the clinical onset of Alzheimer's disease (Mattsson *et al.*, 2016*b*; Olsson *et al.*, 2016). These proteins can also be measured more quickly and less invasively in serum and plasma. Previous studies have documented the association of plasma amyloid-β_{42} levels and the amyloid-β_{42}/amyloid-β_{40} ratio with cognition and risk of all-cause as well as Alzheimer's disease dementia (van Oijen *et al.*, 2006; Seppala *et al.*, 2010; Fandos *et al.*, 2017; Nakamura *et al.*, 2018). In addition, total-tau plasma levels have been shown to improve the prediction of dementia and are suggested as a potential biomarker for risk stratification in prevention trials (Mielke *et al.*, 2018; Pase *et al.*, 2019). In parallel, elevated plasma levels of NfL were found to be associated with neurodegeneration in Alzheimer's disease patients (Mattsson *et al.*, 2017, 2019) and in people at genetically determined risk for Alzheimer's disease compared to control subjects, even before the onset of symptoms (Preische *et al.*, 2019). The repeated measurements of NfL levels over time have also been demonstrated to distinguish mutation carriers of familial Alzheimer's disease from non-carriers earlier compared to single plasma levels of NfL obtained at baseline (Preische *et al.*, 2019). Although previous studies have confirmed tau, amyloid-β and NfL as potentially valuable plasma biomarkers for Alzheimer's disease, a head-to-head comparison of the long-term association with all-cause and Alzheimer's disease dementia in the general population for each individual biomarker and their combinations is currently lacking. Moreover, a longitudinal approach with repeated measurements of the biomarkers in large numbers of healthy individuals at risk from the start of disease incubation and during the long preclinical phase until clinically manifested Alzheimer's disease is needed to assess the robustness of the biomarkers over time.

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In the present study, we first investigated whether plasma concentrations of total-tau, NfL, amyloid-β_{40}, amyloid-β_{42} and the amyloid-β_{42}/amyloid-β_{40} ratio are associated with incident all-cause dementia and Alzheimer's disease dementia in the prospective population-based Rotterdam Study cohort. Second, we quantified the change and course of biomarker plasma levels over time in participants who developed Alzheimer's disease dementia after baseline and compared the results to the age and sex-matched participants who remained dementia-free during up to 14 years follow-up.

Materials and methods

Study design and participants

This study was embedded in the Rotterdam Study, a large population-based cohort designed to ascertain the occurrence and determinants of age-related diseases in the general population (Ikram *et al.*, 2017). In 1989 and 1990, all inhabitants aged 55 and older from a well-defined suburb (Ommoord district) in the city of Rotterdam, the Netherlands, were invited to participate. This first study wave (RS-I) compromised 7983 participants. In 2000, a second study wave (RS-II) of 3011 participants who had reached 55 years of age or moved into the study district since the start of the study, if aged 55 years and older, were added to the first study wave. In 2006, a third study wave (RS-III) of the cohort was initiated, in which 3932 participants were included, aged 45 years and older. In total, the Rotterdam Study comprises 14 926 participants aged 45 years or over (Ikram *et al.*, 2017). The Rotterdam Study has medical ethics committee approval per the Population Study Act Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all participants.

The baseline to study associations of individual biomarker levels with risk of all-cause dementia and of Alzheimer's disease dementia only was defined by the availability of −80°C stored plasma samples obtained from participants during the fourth visit of RS-I between January 2002 and July 2004 and the second visit of RS-II between July 2002 and December 2005. From this selection of 5094 participants, with plasma samples available and informed consent to access medical records during follow-up, we excluded 55 participants without follow-up after this baseline, 77 participants diagnosed with dementia at the baseline visit and 518 participants with one or more missing or invalid test results for plasma levels of biomarkers (for more details see 'Measurement of plasma concentrations of biomarkers' section). As a result, the final sample included 4444 participants for incident dementia analyses, of whom demographic and clinical data were recorded at baseline and during follow-up (Supplementary Table 1). For more details regarding the number of included participants, see Supplementary Fig. 1.

For the analyses of biomarker trajectories and risk of Alzheimer's disease dementia, we compared changes in plasma levels of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} over time of participants who were diagnosed with Alzheimer's disease (cases) to those participants who remained dementia-free during follow-up (controls). Each Alzheimer's disease case (with known time to disease onset) was matched with a dementia-free control of the same sex and age (±3 years). The alignment of plasma measurements of each dementia-free control was anchored to the time to Alzheimer's disease onset of the matched case (for more details, refer to 'Trajectories of biomarkers' section), hence it was particularly important to match cases and control subjects in a one-to-one manner based on age, as the best predictor of time to Alzheimer's disease (Licher *et al.*, 2018).

Measurement of plasma concentrations of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42}

EDTA plasma was sampled, aliquoted and frozen at −80°C according to standard procedures. Measurements were carried out in two separate batches (5094 samples); the first batch included 2000 samples, obtained from a random selection of 1000 participants from the fourth visit of RS-I and 1000 from the second visit of RS-II. The second batch included 3094 samples from the remaining participants of these two study waves. All measurements were performed at Quanterix on a single molecule array (Simoa) HD-1 analyser platform (Rissin *et al.*, 2010). NfL was measured by using the Simoa NF-light® advantage kit (Gisslen *et al.*, 2016; Kuhle *et al.*, 2016; Rohrer *et al.*, 2016). The Simoa Human Neurology 3-Plex A assay (N3PA) was used to measure the concentration of total-tau, amyloid-β_{40} and amyloid-β_{42} (Chang *et al.*, 2017). Samples were tested in duplicate. Two quality control samples were run on each plate for each analyte. When duplicates or single measurements were missing (Supplementary Table 2) or in the case the concentration coefficient of variation exceeded 20% or control samples were out of range (Supplementary Table 3), participant's data were excluded from the analyses.

Ascertainment of dementia and Alzheimer's disease

Dementia screening and diagnosis in the Rotterdam Study has been described elsewhere (de Bruijn *et al.*, 2015). In brief, participants were screened for dementia at baseline and subsequent centre visits with the Mini-Mental State Examination (MMSE) and the Geriatric Mental Schedule organic level. Those with a MMSE score <26 or Geriatric Mental Schedule score >0 underwent further investigation and informant interview, including the Cambridge Examination for Mental Disorders of the Elderly. All participants also underwent routine cognitive assessment. In addition, the entire cohort was continuously under surveillance for dementia through electronic linkage of the study database with medical records from general practitioners and the regional institute for outpatient mental healthcare. Available information on cognitive testing and clinical neuroimaging was used when required for diagnosis of dementia subtype. A consensus panel led by a consultant neurologist established the final diagnosis according to standard criteria for dementia (DSM-III-R), Alzheimer's disease (NINCDS–ADRDA) and vascular dementia (NINDS-AIREN) (Licher *et al.*, 2019). Follow-up until 1 January 2016 was virtually complete (95.7% of potential person-years). Within this period, participants were censored at date of dementia diagnosis, death, loss to follow-up, or 1 January 2016, whichever came first. Detailed information regarding the assessment of dementia subtypes, mild cognitive impairment (MCI) and covariates are described in the Supplementary material.

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Statistical analysis

Descriptive statistics were used to summarize baseline characteristics of all included participants. Means (standard deviation, SD), and counts with percentages, were used to report continuous and discrete variables at the baseline visit, respectively. Plasma concentration (pg/ml) of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} were $\log_2$ transformed (confirmed by Box-Cox screening, see Supplementary material) for further analysis to comply with the assumption of normality in the distribution of the data. Pearson correlation and linear regression were used to explore the relationship between plasma levels and age.

Associations of baseline levels of each biomarker with risk of all-cause and Alzheimer's disease dementia

Dementia was considered as the primary study end point. Plasma total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} were analysed on a continuous scale and according to quartiles. In each of the two assay batches the plasma concentration for each individual protein was ranked and divided into four equally sized groups. Group Q1 representing the group with the lowest concentration, Q2 the second lowest group, Q3 the second highest group and Q4 the highest group. For each protein the groups for both batches were then combined into one variable (of four equally sized groups). In standard Cox proportional hazard regression models, time was modelled as follow-up time after baseline. The proportional hazard assumption was tested by including time-dependent interactions between time after baseline and total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} plasma levels and the amyloid-β_{40}/amyloid-β_{42} ratio (all $\log_2$ transformed) on the risk for all-cause and Alzheimer's disease dementia. We used a linear interaction between time and plasma proteins. In sensitivity analyses, we repeated the biomarker analyses among cognitively unimpaired participants, by excluding participants with MMSE score <26 or an MCI diagnosis at baseline, to evaluate whether plasma protein levels were still associated with development of all-cause and of Alzheimer's disease dementia. All analyses were repeated without adjusting for batch number. We used the cause-specific cumulative incidence function to translate results to absolute risks for all-cause and Alzheimer's disease dementia according to age for each of the marker quartile groups. To overcome issues of competing risks, we analysed the data considering the occurrence of competing events by computing cumulative incidence as left truncated data, with age as the time scale. Observation time started at the age of participants at the date of sampling and ended at the age of diagnosis of all-cause or Alzheimer's disease dementia, death, loss to follow-up or end of the study, whichever came first (Klein and Moeschberger, 2003; Thiebaut and Benichou, 2004). Death and dementia other than Alzheimer's disease were treated as competing events for Alzheimer's disease analysis (Fine and Gray, 1999).

Associations of combined biomarkers with risk of all-cause and Alzheimer's disease dementia

We investigated the association of combined plasma protein levels with all-cause and Alzheimer's disease dementia by including an interaction term for the quartile group analysis as well as the analysis on a continuous scale. Significance of multiplicative interaction on a continuous scale and using the four equally sized groups, was tested using the χ^2 likelihood test using Wilk's approximation.

For both the individual plasma proteins as well as for their combinations, we used the following models for adjustment. In a first model (Model I), we adjusted for age (using a linear and a quadratic term), sex, and assay batch number. In a second model (Model II), we further adjusted for baseline educational level, cardiovascular risk factors [smoking status, systolic blood pressure, total and high-density lipoprotein (HDL) cholesterol, body mass index, history of diabetes, and stroke or coronary heart disease] and *APOE* ε4 status (i.e. presence of 0, 1 or 2 *APOE* ε4 alleles). Measurements of systolic blood pressure and total and HDL cholesterol were missing in three, three and 12 participants, respectively, in which case we imputed the mean, conditional on age and sex. In a third model (Model III), we additionally adjusted for plasma levels of all other proteins.

Trajectories of biomarkers

Linear mixed models with random intercepts and slopes were used to estimate trajectories of $\log_2$ plasma levels of total-tau, NfL, amyloid-β_{40}, amyloid-β_{42} on a population level for those who developed Alzheimer's disease dementia (cases) and the age- and sex-matched participants who remained dementia-free (controls) during follow-up. Follow-up times were calculated by subtracting the time of plasma measurement from the date of diagnosis for those who developed Alzheimer's disease. As a result, negative follow-up times represent the time (in years) before Alzheimer's disease onset, whereas positive follow-up times represent the time (in years) after the disease onset. For the dementia-free controls (for whom, by definition the date of diagnosis is not observed) an index date identical to the date of diagnosis of the corresponding matching case was used. We included age at baseline for baseline measurements and age at visiting the research centre for follow-up measurements and sex in a primary model, and additionally extended this model with educational level and *APOE* ε4 carrier status. As a complementary analysis, we assessed the rate of change of Alzheimer's disease-associated proteins on an individual level to examine non-linearity as a function of disease progression among those with two or more biomarker measurements. A rate ('slope') of change for each individual was extracted from a linear mixed effects model, which included an interaction term between time and case/control to allow different rates for cases compared to controls over time. Individual rates of change were then regressed on time from the first measurement taken in cases or controls to conversion or index-date, respectively. In addition, we modelled the trajectories of Alzheimer's disease-associated plasma protein levels on age as time scale. In this analysis, we adjusted for all of the covariates, and instead of time to diagnosis used age of visiting the centre as timescale.

All analyses were done at the significance level of 0.05 (two-tailed) using SAS version 9.4, SPSS Statistics version 24.0.0.1 (IBM Corp., Armonk, NY) and R version 3.4.3, using the 'survival', 'npsurv', 'rstan', 'lme4' and 'rms' packages.

Data availability

The data that support the findings of this study are available on request from the corresponding author, subject to local and European regulations.

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Results

Baseline characteristics

A total of 4444 participants were included in the analysis for the risk of dementia with an average age at baseline of 71.9 (SD 7.5) years, 2555 (57.5%) participants were female (Table 1). The highest level of attained education varied, with 11.1% having only attained primary education and 43.5% with a lower/intermediate general education or lower vocational education. The median baseline MMSE score was 28 [interquartile range (IQR) 26–30] and 424 participants (9.5%) had a MMSE score <26. At baseline, 419 participants (9.4%) had MCI and 114 (25.7%) were *APOE* ε4 carriers (homozygous plus heterozygous) (Corder *et al.*, 1993).

Mean baseline plasma levels of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} measured in the 4444 participants were 2.6 (SD 2.3), 16.0 (SD 12.9), 266.0 (SD 55.0) and 10.7 (SD 3.0) pg/ml, respectively (Table 1). NfL and amyloid-β_{40} plasma levels increased with age (Pearson coefficient r = 0.59 and r = 0.38, respectively, all $P < 0.0001$; Fig. 1). Correlations with age were much weaker for total-tau (r = 0.09, $P < 0.0001$) and amyloid-β_{42} plasma levels (r = 0.17, $P < 0.0001$). Plasma levels of amyloid-β_{40} and amyloid-β_{42} were correlated (r = 0.59, $P < 0.0001$, Supplementary Table 4) as was the case for amyloid-β_{42} plasma levels and the plasma amyloid-β_{42}/amyloid-β_{40} ratio (r = 0.67, $P < 0.0001$). Plasma levels of NfL were correlated with those of amyloid-β_{40} (r = 0.49, $P < 0.0001$) and amyloid-β_{42} (r = 0.33, $P < 0.0001$).

During a total follow-up time of 40 577 person-years, 549 individuals developed dementia (12.4%). The median follow-up time from baseline to dementia diagnosis was 6.7 years (IQR: 3.4–9.1 years). Among those, Alzheimer's disease dementia was diagnosed in 374 (68%) participants after a median of 6.6 years (IQR: 2.9–9.0 years). Possible Alzheimer's disease and Alzheimer's disease plus cardiovascular disease were diagnosed in 75 (13.7%) participants and were not included in the analyses with Alzheimer's disease as end-point as was the case for the remaining 100 (18.2%) individuals who were diagnosed with vascular dementia, Parkinson dementia, or an undetermined type of dementia (Supplementary Fig. 1). Median follow-up for those who remained alive and dementia-free was 11.2 years (IQR: 10.8–12.1), with a maximum follow-up of 13.9 years.

Table 1 Baseline characteristics of the participants

Variable	Study population (*n* = 4444)
Age	71.9 (7.5)
Female	2555 (58)
MMSE[a]	27.8 (2)
⩽20	30 (1)
21–25	394 (9)
26–29	3328 (75)
30	652 (15)
MCI[a]	419 (9)
Education level	
Primary education	493 (11)
Lower-intermediate education level	1931 (44)
Intermediate-higher level	1342 (30)
Higher-university education level	614 (14)
APOE ε4	
0 alleles	3083 (69)
1 allele	1066 (24)
2 alleles	77 (2)
Smoking	
Never	1306 (29)
Former	2379 (54)
Current	673 (15)
Body mass index, kg/m^2	27.6 (4)
<18.5	23 (1)
18.5–25.0	1132 (26)
25.0–30.0	2156 (49)
30.0–35.0	827 (19)
>35.0	216 (5)
Total cholesterol, mmol/l	5.6 (1)
HDL cholesterol, mmol/l	1.5 (0.4)
Systolic blood pressure, mmHg	149.1 (21.1)
Diastolic blood pressure, mmHg	79.7 (10.9)
Coronary heart disease	431 (10)
Diabetes	581 (14)
Stroke	197 (4)
Biomarker plasma levels, pg/ml, mean (SD), IQR [min, max]	
Total-tau	2.6 (2.3), 1.9–3.0 [0.4, 141.4]
NfL	16.0 (12.9), 10.0–18.3 [3.4, 390.8]
Amyloid-β_{40}	266.0 (55.0), 230.5–294.1 [52.4, 614.6]
Amyloid-β_{42}	10.7 (3.0), 8.9–12.1 [0.5, 94.2]

Non-imputed data presented as frequency (%) for categorical values and mean ± SD for continuous variables, unless indicated otherwise. Data at baseline were virtually complete (<5.0% missing).
[a]For ascertainment of MCI, see Supplementary material.
HDL = high-density lipoprotein; MMSE = Mini-Mental State Examination.

Association of plasma biomarkers with risk of all-cause and Alzheimer's disease dementia

A $\log_2$ higher concentration of NfL in plasma measured at baseline was associated with an increased risk for developing all-cause dementia {hazard ratio (HR) 1.54 [95% confidence interval (CI), 1.34–1.75]; $P < 0.0001$}, and for Alzheimer's disease dementia [HR 1.49 (95% CI, 1.27–1.74); $P < 0.0001$], while adjusting for age, sex and assay batch number (Model I; Table 2). In the same model, a $\log_2$ higher amyloid-β_{42} concentration in plasma was associated with a decreased risk for all-cause dementia [HR 0.60 (95% CI, 0.49–0.74); $P < 0.0001$] and for Alzheimer's disease dementia [HR 0.60 (95% CI, 0.47–0.76); $P < 0.0001$]. After further adjustment for all other biomarkers, educational level, cardiovascular risk factors and *APOE* ε4 status (Model III), the association between development of all-cause dementia and a $\log_2$ increase of baseline plasma NfL [HR 1.59 (95% CI, 1.38–1.83); $P < 0.0001$] and amyloid-β_{42} [HR 0.61

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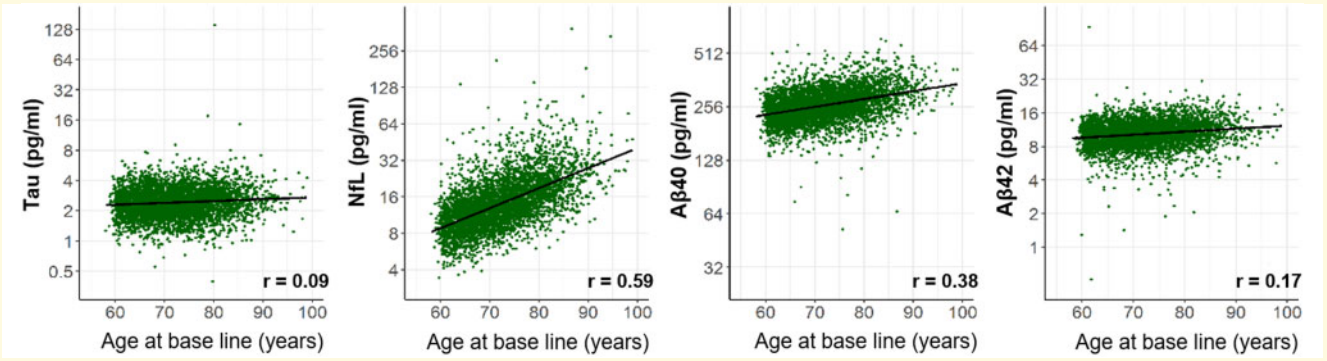


Figure 1 Correlation of plasma levels of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} with age. The correlation between individual plasma total-tau, NfL, amyloid-β_{40} (Aβ40), and amyloid-β_{42} (Aβ42) levels (pg/ml) with age are shown. The black line represents the mean obtained by regressing each biomarker on age. r = Pearson correlation coefficient; *P*-value for each biomarker <0.0001.

Table 2 Association of plasma levels of total-tau, NfL, amyloid-β_{40}, amyloid-β_{42} and the amyloid-β_{42}/amyloid-β_{40} ratio with all-cause dementia

Biomarker	Association with all-cause dementia					
	Model I		Model II		Model III	
	HR (95%CI)	*P*-value (overall)	HR (95%CI)	*P*-value (overall)	HR (95%CI)	*P*-value (overall)
Tau (per $\log_2$ pg/ml increase)	1.20 (1.00, 1.45)	0.049	1.16 (0.96, 1.40)	0.1333	1.16 (0.95, 1.41)	0.13771
NfL (per $\log_2$ pg/ml increase)	1.54 (1.34, 1.75)	<0.0001	1.50 (1.31, 1.71)	<0.0001	1.59 (1.38, 1.83)	<0.0001
Amyloid-β_{40} (per $\log_2$ pg/ml increase)	0.87 (0.64, 1.19)	0.394	0.84 (0.62, 1.16)	0.2995	0.86 (0.58, 1.29)	0.47899
Amyloid-β_{42} (per $\log_2$ pg/ml increase)	0.60 (0.49, 0.74)	<0.0001	0.66 (0.53, 0.83)	0.0002	0.61 (0.47, 0.78)	<0.0001
Amyloid-β_{42}/amyloid-β_{40} ratio[a]	0.53 (0.42–0.68)	<0.0001	0.62 (0.47, 0.80)	0.0004	0.63 (0.48-0.82)	0.00052

Model I: Adjusted for age, sex and assay batch number.
Model II: Model I + additional adjustment for highest level of education, smoking status, systolic blood pressure, total and high-density lipoprotein cholesterol, body mass index, history of diabetes, stroke and coronary heart disease, and *APOE* ε4 status.
Model III: Model II + additional adjustment for plasma levels of other biomarkers.
[a]Model III for amyloid-β_{42}/amyloid-β_{40} ratio: Model II + additional adjustment only for plasma levels of total-tau and NfL.

(95% CI, 0.47–0.78); $P < 0.0001$] remained nearly identical (Table 2). Similar patterns were observed for Alzheimer's disease dementia (Supplementary Table 5). In addition, baseline $\log_2$ amyloid-β_{42}/amyloid-β_{40} ratios were significantly associated with risk of developing all-cause and Alzheimer's disease dementia in the first, age, sex and batch adjusted analysis [HR 0.53 (95% CI, 0.42–0.68) and HR 0.52 (95% CI, 0.39–0.70), respectively; $P < 0.0001$] and after further adjustment in Model III [HR 0.63 (95% CI, 0.48–0.82) and HR 0.62 (95% CI, 0.45–0.85), respectively; $P < 0.005$]. In contrast, baseline total-tau and amyloid-β_{40} plasma levels were not significantly associated with risk for all-cause or Alzheimer's disease dementia (Table 2 and Supplementary Table 5). Repeating the analyses without adjusting for batch number did not meaningfully change effect estimates (Supplementary Table 6). Associations of plasma levels of biomarkers with other types of dementia are presented in Supplementary Tables 7 and 8, showing increase of baseline NfL levels to be associated with vascular dementia [HR 1.95 (95% CI, 1.19–3.20); $P = 0.008$] and with other subtypes of non-Alzheimer's disease dementia [HR 1.78 (95% CI, 1.18–2.68); $P = 0.006$].

To evaluate whether plasma levels of NfL and amyloid-β_{42} were still associated with development of all-cause and Alzheimer's disease dementia in cognitively unimpaired participants at baseline, we repeated the analysis excluding 742 (16.7%) individuals who had a baseline MMSE score of <26 or a MCI diagnosis from the analysis. Of the remaining 3702 participants, 357 developed dementia including 239 cases of Alzheimer's disease dementia. The hazard ratio for all-cause dementia per $\log_2$ increase of NfL was 1.70 (95% CI, 1.40–2.06) and for amyloid-β_{42} was 0.56 (95% CI, 0.40–0.76). Similar results were found for Alzheimer's disease, with a hazard ratio per $\log_2$ increase of NfL of 1.64 (95% CI, 1.29–2.09) and for amyloid-β_{42} of 0.60 (95% CI, 0.41–0.89), which are very similar to those obtained from the main analyses.

Next, we ranked the baseline plasma levels of total-tau, NfL, amyloid-β_{40}, amyloid-β_{42} and amyloid-β_{42}/amyloid-β_{40} ratio and divided them according to quartiles into four equally sized groups (Q1, Q2, Q3, Q4 with Q1 the group with the lowest plasma concentrations) and compared Q2, Q3 and Q4 to Q1 as reference. The risk for all-cause dementia showed a gradual increase per quartile group of plasma NfL

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($P < 0.0001$) with a hazard ratio of 2.70 (95% CI, 1.85–3.85) for Q4 compared to Q1 (Fig. 2A). Similar patterns were found for Alzheimer's disease [HR 3.28 (95% CI, 2.05–5.24); $P < 0.0001$] (Supplementary Fig. 2). Moreover, the risk for all-cause and Alzheimer's disease dementia significantly decreased per quartile group of amyloid-β_{42} ($P < 0.0001$ for both). Although there was a significant association between quartile groups of total-tau and the risk of all-cause and Alzheimer's disease dementia ($P = 0.003$ and 0.005, respectively), trends were not consistent (Fig. 2A and Supplementary Fig. 2). Quartile groups of increasing amyloid-β_{40} were not significantly associated with all-cause dementia ($P = 0.07$) or Alzheimer's disease dementia ($P = 0.12$). Repeating the analyses without adjusting for batch number did not meaningfully change the effect estimates (Supplementary Table 9).

Plasma biomarker levels and cumulative incidence for all-cause and Alzheimer's disease dementia

Dementia risk profiles according to age for the four quartile groups of baseline plasma levels of total-tau, NfL, amyloid-β_{40}, amyloid-β_{42} and amyloid-β_{42}/amyloid-β_{40} ratio are shown in Fig. 2B. For total-tau, as well as for amyloid-β_{40}, curves overlapped indicating that plasma levels were not linearly associated with the development of all-cause dementia. For NfL, the risk for all-cause dementia increased with each quartile group, whereas for amyloid-β_{42} the risk decreased with each quartile group (Fig. 2B). We repeated the analyses for Alzheimer's disease dementia and observed similar patterns (Supplementary Fig. 2). We then investigated if the hazard ratio for all-cause and for Alzheimer's disease dementia at baseline remained constant over the course of follow-up by including a linear interaction with follow-up time and plasma biomarkers in the model (Supplementary Table 10). For NfL, the hazard ratio for Alzheimer's disease declined significantly ($P = 0.003$) from 2.29 (95% CI, 1.77–2.95) per $\log_2$ NfL higher at baseline to 1.18 (95% CI, 0.88–1.57) 10 years thereafter. The hazard ratio for Alzheimer's disease per $\log_2$ higher amyloid-β_{42} level did not change significantly over time ($P = 0.35$), nor did the hazard ratio for total-tau and amyloid-β_{40} ($P = 0.70$ and $P = 0.86$, respectively).

Association of combined plasma levels of NfL and amyloid-β_{42} with risk of all-cause and Alzheimer's disease dementia

We observed that 41 (30.4%) of the 135 participants in the highest (Q4) quartile group for NfL combined with the lowest (Q1) quartile group of amyloid-β_{42} developed all-cause dementia, 28 with Alzheimer's disease dementia. In contrast, only two (1.9%) incident cases of all-cause dementia were found in the 107 participants in the lowest (Q1) quartile group for NfL in combination with highest (Q4) quartile group for amyloid-β_{42} (Supplementary Table 11). The hazard ratio for developing all-cause dementia was 9.5 (95% CI, 2.3–40.4; $P = 0.0022$), and for Alzheimer's disease dementia was 15.7 (95% CI, 2.1–117.4; $P < 0.0001$), when the highest quartile group of NfL was combined with the lowest quartile group of amyloid-β_{42} and compared to the combination of lowest quartile group of NfL and highest quartile group amyloid-β_{42} (Fig. 3). NfL and amyloid-β_{42} were each independently associated with the risk of developing all-cause dementia and Alzheimer's disease dementia as tests for interaction between NfL and amyloid-β_{42} were not significant ($P = 0.15$ and $P = 0.34$, respectively, when NfL and amyloid-β_{42} were modelled on the continuous $\log_2$ scale). However, confidence intervals of hazard ratios for each of the 16 quartile NfL–amyloid-β_{42} combinations were wide because of the smaller numbers in each combination.

Plasma NfL and amyloid-β_{42} trajectories during the development of Alzheimer's disease dementia

As results for all-cause dementia and the largest subgroup of Alzheimer's disease dementia were shown to be nearly identical, we focused on the latter for our study on changes in plasma concentrations of biomarkers, in particular NfL and amyloid-β_{42}, over time. Mean trajectories and 95% CIs for plasma NfL and amyloid-β_{42} levels, along with their corresponding individual-level rates of change, were compared between the 374 participants who developed Alzheimer's disease dementia and the 374 age- and sex-matched controls who remained dementia-free during the follow-up time (Fig. 4). Plots of other biomarkers are shown in Supplementary Fig. 3. Participants contributed one to three plasma measurements over time.

Plasma NfL increased linearly for both Alzheimer's disease dementia cases and dementia-free controls (Fig. 4A). The change of NfL per year was 3.4 times faster for participants who developed Alzheimer's disease dementia [$\log_2$ 0.04 pg/ml (95% CI, 0.02–0.06)] compared to those who did not [$\log_2$ 0.01 pg/ml (95% CI, 0.00–0.02), P for interaction < 0.0001]. A significant difference in the rate of change between cases and controls was also found in the individual-level rate of change analysis among those with two or more measurements ($P < 0.0001$). Absolute plasma NfL levels in participants who developed Alzheimer's disease dementia were estimated to start at 14.62 pg/ml (95% CI, 13.33–16.03) 13 years before diagnosis compared to 15.93 pg/ml (95% CI, 14.63–17.35) for dementia-free control subjects. A subsequent faster rate of change for cases led to a higher absolute value of NfL at the time of Alzheimer's disease dementia diagnosis, 20.77 pg/ml (95% CI, 19.21–22.42) compared to 17.66 pg/ml (95% CI, 16.50–18.93) for controls at the time of index date, and 13 years thereafter 29.49 pg/ml (95% CI, 25.22–34.47) for cases compared to 19.60 pg/ml (95% CI, 17.53–21.93) for control subjects.

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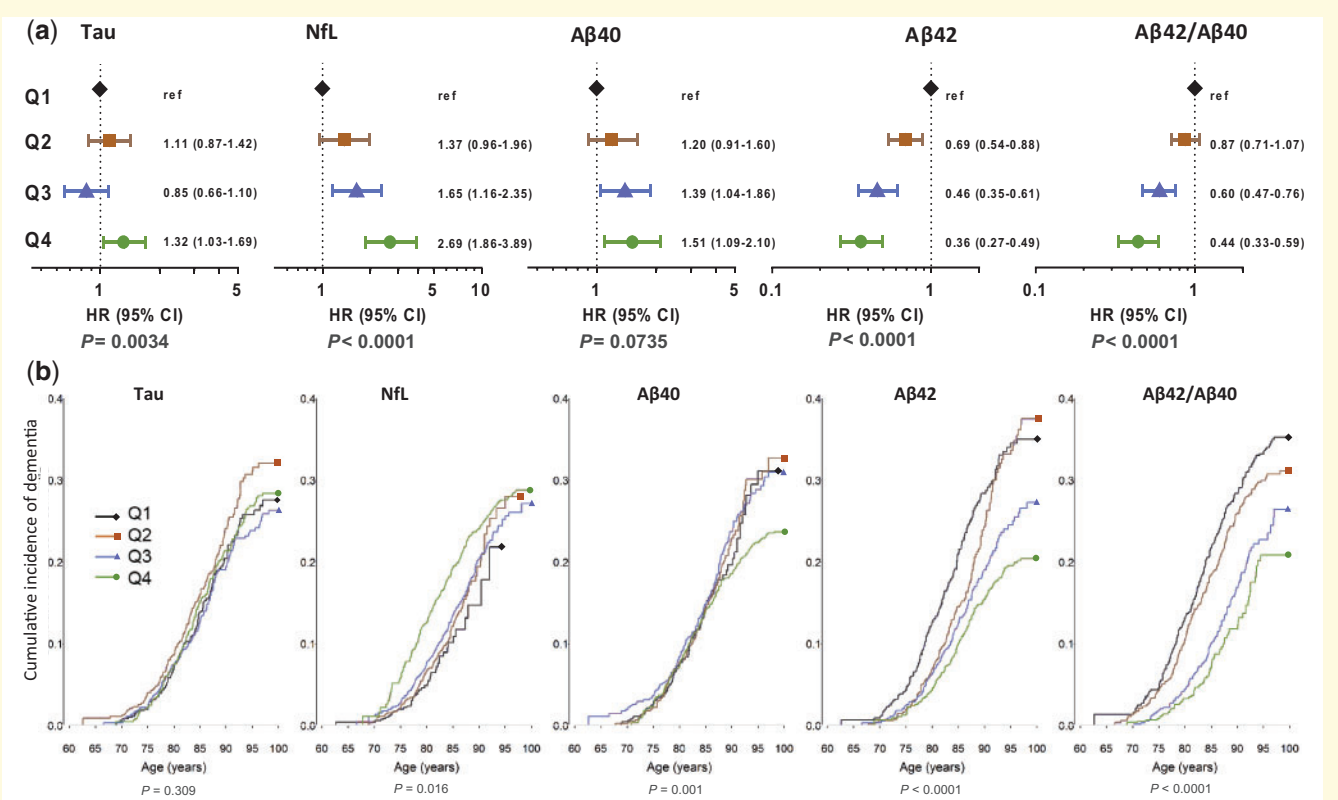


Figure 2 Association of plasma total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} levels and amyloid-β_{42}/amyloid-β_{40} ratio with all-cause dementia. (**A**) Forest plots showing hazard ratios for all-cause dementia and 95% CI per quartile of plasma levels (pg/ml) of total-tau, NfL, amyloid-β_{40}, amyloid-β_{42}, and amyloid-β_{42}/amyloid-β_{40} ratio, with the lowest quartile (Q1) as reference group. Hazard ratios were obtained from the Cox proportional hazard models that were adjusted for age, sex, assay batch number, systolic blood pressure, total and HDL cholesterol, smoking status, highest level of education, body mass index, *APOE ε4* status, history of diabetes, stroke and coronary heart disease. (**B**) Cause-specific incidence curves showing the incidence of all-cause dementia with current age for total-tau. *P*-value for test for equality of the cause-specific cumulative incidence curve between the five groups: total-tau ($P = 0.3087$), NfL ($P = 0.0162$), amyloid-β_{40} ($P = 0.0012$), amyloid-β_{42} ($P < 0.0001$) and amyloid-β_{42}/amyloid-β_{40} ratio ($P < 0.0001$) (Klein and Moeschberger, 2003). Plasma levels are categorized into equally sized groups using quartiles (lowest group, Q1: black, Q2: brown, Q3: blue and Q4: green line). In total, 549 individuals had a diagnosis of all-cause dementia and 1229 individuals had death as a competing event. The remaining 2666 individuals were censored. Numbers represent the total number per quartile at risk for all-cause dementia at a given age.

The mean plasma NfL concentration found over time in the Alzheimer's disease cases began to deviate from the mean concentration found in the dementia-free controls at 9.6 years prior to Alzheimer's disease diagnosis or index date (Fig. 4A). On an age scale, absolute plasma NfL levels in the participants who developed Alzheimer's disease began to deviate from those that remained dementia-free from a mean age of 60.3 years on (Supplementary Fig. 4). Furthermore, the effect of time on the rate of change of plasma NfL did not reach statistical significance (Fig. 4C), indicating that the changes in NfL plasma levels do not accelerate or decelerate over time.

In those who developed Alzheimer's disease dementia and those who did not, mean plasma amyloid-β_{42} levels declined and their rates of decline were not significantly different [$\log_2$ –0.01 pg/ml per year (95% CI, –0.02, 0.00) for cases and (95% CI, –0.01, 0.00) for controls, $P = 0.189$] (Fig. 4B). On average, plasma amyloid-β_{42} levels in participants who developed Alzheimer's disease dementia declined from 11.03 (95% CI, 10.47–11.62) around 13 years before diagnosis to 10.08 (95% CI, 9.66–10.53) at the time of diagnosis. In control subjects, amyloid-β_{42} levels declined from 11.10 (95% CI, 10.63–11.58) to 10.61 (95% CI, 10.29–10.96) over the same period. After diagnosis or index date, the levels declined further to 9.22 (95% CI, 8.37–10.15) for cases and 10.15 (95% CI, 9.52–10.81) for control subjects. A significant ($P = 0.038$) difference was found at the individual-level rate of change in those who developed Alzheimer's disease dementia and those who did not, however, the effect size was negligible (Fig. 4D). As with plasma NfL, there was no evidence of non-linearity in the changes of amyloid-β_{42} over time.

In parallel analyses on an age scale, we observed significantly different effects of increasing age on plasma NfL levels between cases with Alzheimer's disease dementia and dementia-free control subjects for amyloid-β_{40} and amyloid-

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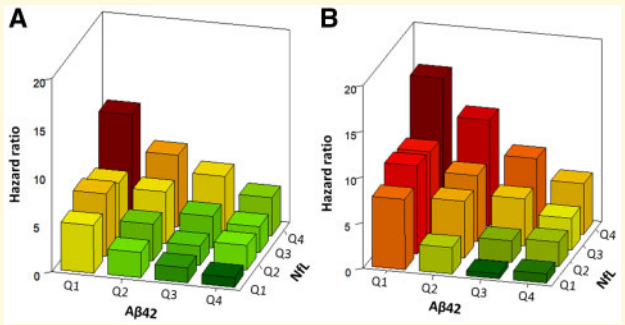


Figure 3 Hazard ratios for all-cause and Alzheimer's disease dementia per quartile of plasma amyloid-β_{42} and NfL combined. Bars show the hazard ratios (HRs) for all-cause dementia (**A**) and for Alzheimer's disease dementia (**B**) per quartile of plasma NfL and amyloid-β_{42}, with the combination of the highest quartile group (Q1) of amyloid-β_{42} and the lowest quartile group (Q4) of NfL as reference group (HR = 1.0). Hazard ratios were obtained with the Cox proportional hazard model adjusted for age, sex, assay batch number, highest level of education, smoking status, systolic blood pressure, total and HDL cholesterol, body mass index, history of diabetes, stroke and coronary heart disease and *APOE ε4* status. There were no significant interactions between amyloid-β_{42} and NfL for all-cause dementia ($P = 0.15$) and for Alzheimer's disease dementia ($P = 0.32$), suggesting that the association between amyloid-β_{42}, NfL and the risk of dementia are independent of each other.

β_{42}/amyloid-β_{40} ratio ($P = 0.010$ and $P = 0.045$, respectively), although absolute differences were negligible (Supplementary Fig. 4). The effects of increasing age on plasma total-tau and amyloid-β_{42} levels were not significant (both $P > 0.5$) (Supplementary Fig. 4).

Discussion

Within this large prospective population-based cohort study, we investigated whether levels of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} and the amyloid-β_{42}/amyloid-β_{40} ratio measured in plasma of non-demented participants at baseline were associated with incident all-cause dementia and with Alzheimer's disease dementia. We demonstrated that independent of age, sex, education, cardiovascular risk factors and *APOE* ε4 carrier status, high plasma levels of NfL and low plasma levels of amyloid-β_{42} are each independently associated with an increased risk of developing all-cause and Alzheimer's disease dementia during 13.9 years of follow-up. When combined, high plasma NfL and low plasma amyloid-β_{42} levels showed an even stronger association with risk for all-cause as well as Alzheimer's disease dementia. Our analysis of the rate of changes in plasma levels of biomarkers further showed a stable increase of NfL in those who developed Alzheimer's disease dementia, where plasma levels begin to deviate from the levels found in those who remained dementia-free from ~10 years prior to Alzheimer's disease diagnosis. Plasma levels of total-tau did not show a consistent association with risk for all-cause or Alzheimer's disease dementia and trajectories found in participants who developed Alzheimer's disease were also not different from those who did not. Amyloid-β_{40} levels increased with age but were not associated with all-cause or Alzheimer's disease dementia risk.

Associations of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} levels in CSF with Alzheimer's disease have been established in several studies (Mattsson *et al.*, 2016*a*; Olsson *et al.*, 2016). In their meta-analysis, for example, Olsson and colleagues (2016) showed that high levels of tau and NfL and low levels of amyloid-β_{42} in CSF are strongly associated with clinically diagnosed Alzheimer's disease. We reported previously that high amyloid-β_{40} in combination with low amyloid-β_{42} plasma levels are associated with risk for Alzheimer's disease (van Oijen *et al.*, 2006; Hilal *et al.*, 2018). In the present study, we found that higher NfL and lower amyloid-β_{42} plasma levels are significantly associated with incident all-cause and Alzheimer's disease dementia. We did not find a clear association between plasma total-tau levels and all-cause or Alzheimer's disease dementia risk, which is in line with results from a study amongst memory clinic patients recently reported by Verberk *et al.* (2018). In contrast, Mattsson *et al.* (2016*b*) found plasma tau to be significantly associated with worse cognition, atrophy and hypometabolism during follow-up in the ADNI study. Association of plasma total-tau with incident all-cause dementia was also reported from the participants of the Framingham Heart Study (Pase *et al.*, 2019). These contradictory results may signal that plasma total-tau is a non-specific marker (Deters *et al.*, 2017; Jack *et al.*, 2018; Pase *et al.*, 2019) or a relatively late marker for the risk of developing Alzheimer's disease (Mattsson *et al.*, 2016*b*). In addition, on the individual level, CSF and plasma tau did not show a strong correlation (Mattsson *et al.*, 2016*a*), possibly reflecting peripheral degradation of total-tau, rendering tau fragments undetectable in the assay used. This effect may also explain our finding that plasma total-tau is not associated with risk for all-cause and Alzheimer's disease dementia.

Our findings on the association between plasma levels of NfL in a dementia-free population and development of all-cause and Alzheimer's disease dementia are in line with cross-sectional studies done in ADNI (Mattsson *et al.*, 2017; Zhou *et al.*, 2017), where significant differences were found between Alzheimer's disease and cognitively normal control subjects, but also between control subjects and MCI, indicating that the plasma NfL level is altered in a stage of Alzheimer's disease where early clinical symptoms and cognitive decline become noticeable. We also found that the association between NfL and Alzheimer's disease dementia is strongest for shorter follow-up and gradually declines as the duration of follow-up increases. The results from our longitudinal analysis that NfL plasma levels start to increase ~10 years before Alzheimer's disease diagnosis appear to confirm this. These results may indicate that NfL is a marker of progressive myelinated axonal damage that develops in the mid- to late stage of the preclinical phase of Alzheimer's disease

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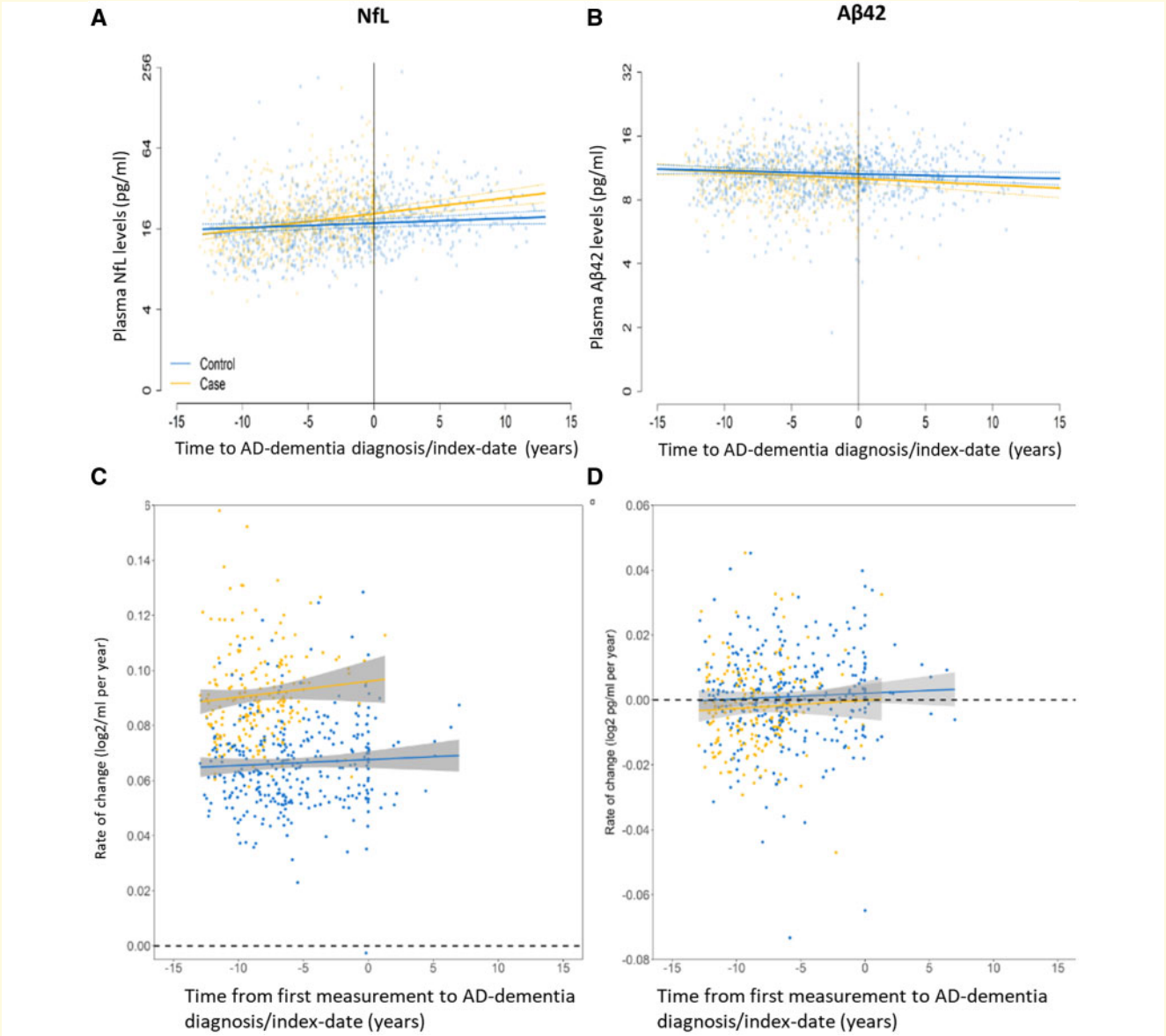


Figure 4 Longitudinal changes of plasma NfL and amyloid-β_{42} levels and their rates of change over time before and after Alzheimer's disease dementia diagnosis. (**A**) Trajectories of plasma NfL levels on a population-level are shown for Alzheimer's disease (AD) dementia cases and dementia-free control subjects. (**B**) Trajectories of plasma amyloid-β_{42} levels are shown for Alzheimer's disease dementia cases and dementia-free control subjects. The yellow and blue lines depict estimated means of plasma levels based on repeatedly measured biomarkers for 374 cases with Alzheimer's disease dementia and their age- and sex-matched controls, respectively. Dotted lines represent 95% CI. Background shaded dots are coloured accordingly and denote single measurements corresponding to data where models were fit upon. (**C**) Rate of change of plasma NfL at the individual level for cases with Alzheimer's disease dementia compared to dementia-free control subjects. (**D**) Rate of change of plasma amyloid-β_{42} at the individual level for cases with Alzheimer's disease dementia compared to dementia-free control subjects. For these analyses, a $\log_2$-transformed rate of change was estimated for those participants that had two or more biomarker measurements. One data point reflects the rate of change for each of these individuals, anchored at the time since disease diagnosis relative to first biomarker measurement. Analyses with linear mixed effect models were adjusted for age at baseline, sex, *APOE* ε4 status, time between baseline measurement and disease onset and the interaction with case or control status. Rates of change derived from the population level (**A** and **B**) are different compared to those derived from the individual level analysis presented in **C** and **D**, as population level analyses are based on the entire sample, and individual level analysis required multiple measurements and were therefore only carried out among those participants that contributed two or three measurements.

when subjects are incubating the disease (Ikram *et al.*, 2010; Rohrer *et al.*, 2016). Notably, individuals in this stage of disease development respond better to potential therapies for Alzheimer's disease than those with symptomatic disease, suggesting NfL as a promising biomarker to detect both degeneration at earlier stages of Alzheimer's disease and

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response to treatment (Bacioglu *et al.*, 2016; Mattsson *et al.*, 2019). In our study, amyloid-β_{42} plasma levels declined over time in the participants who developed Alzheimer's disease dementia, although the difference was not significant when compared to participants who remained dementia-free during follow-up. This might suggest that amyloid-β_{42} is a marker earlier in Alzheimer's disease incubation. In line with this conjecture, previous studies have postulated that the plasma amyloid-β levels are increased before the onset of sporadic Alzheimer's disease (Seppala *et al.*, 2010; Tijms *et al.*, 2018).

Our results could serve as a basis to investigate further whether baseline plasma NfL levels yield predictive value to identify those asymptomatic individuals that are at significant risk of developing Alzheimer's disease dementia. However, increased CSF and plasma levels of NfL are also observed in other neurodegenerative diseases than Alzheimer's disease (Mattsson *et al.*, 2017). In our study higher plasma NfL, but not amyloid-β_{42}, levels were associated with risk of vascular and other dementias, although absolute numbers were small. When we combined plasma levels of NfL and amyloid-β_{42}, we found a strong association with incident Alzheimer's disease, although the results should be interpreted with caution because of wide confidence intervals due to the small numbers in each of the biomarker combinations. At the same time, this indicates that NfL and amyloid-β_{42} combined may be even more useful in identifying individuals at high risk of Alzheimer's disease.

Finally, our results revealed that the average plasma concentration of NfL in participants who develop Alzheimer's disease dementia rise at a constantly higher rate than in participants who remained dementia-free. On a population level this translates into NfL plasma concentrations in those who develop Alzheimer's disease starting to deviate from those who remain dementia-free at ~10 years before diagnosis of disease. These results may allow for further modelling dynamics of plasma marker levels in addition to those in CSF (Ower *et al.*, 2018), establishing NfL as a stable biomarker while taking into account its plasma variability over time.

The findings of this study should be considered in light of some limitations. First, we were unable to compare results of our measurements in plasma with those in CSF of the same participants, as CSF is not systematically collected in the population-based Rotterdam Study. Second, Alzheimer's disease has been consistently diagnosed according to the clinical NINCDS–ADRDA criteria in the Rotterdam Study, while new frameworks for (preclinical) Alzheimer's disease diagnoses have been introduced, such as the NIA-AA research framework (Jack *et al.*, 2018). Moreover, amyloid PET or CSF were not available in the Rotterdam Study to further confirm the pathological diagnosis of Alzheimer's disease. However, final Alzheimer's disease dementia diagnoses were established via a consensus panel led by a neurologist as is commonly done in large population-based studies (Chibnik *et al.*, 2017). The main strength of our study is that it is based on data obtained from dementia-free participants in a large population-based prospective cohort study with long follow-up. In addition, simultaneous assessment of total-tau, NfL, amyloid-β_{40} and amyloid-β_{42} in plasma repeatedly obtained from single individuals facilitated a head-to-head comparison of the biomarkers to quantify their importance in the association with all-cause and Alzheimer's disease dementia in the general population.

In conclusion, we found that higher levels of NfL and lower levels of amyloid-β_{42} plasma concentrations are each independently and in combination strongly associated with a higher risk of developing all-cause as well as Alzheimer's disease dementia. Repeated measurements over time indicated that increasing NfL levels in plasma may occur as early as 10 years prior to Alzheimer's disease dementia diagnosis. These findings indicate that NfL and amyloid-β_{42} measured in plasma can potentially contribute to assess the risk for dementia while the disease is still in its preclinical stage. Moreover, plasma NfL levels, although not specific, may be useful in monitoring progression of Alzheimer's disease dementia.

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Competing interests

None of the authors has anything to disclose in addition to the sponsorship of the study by Janssen Pharmaceuticals of

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Johnson and Johnson. G.J.W., P.R.W. and J.M.K. are and F.d.W., L.G., W.K. and J.G. were employed by Janssen Pharmaceuticals of Johnson & Johnson.

Supplementary material

Supplementary material is available at *Brain* online.

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