Cerebrospinal fluid biomarkers of neurodegeneration, synaptic integrity and astroglial activation across the clinical Alzheimer's disease spectrum

Isabelle Bos[1]*, Stephanie Vos[1], Frans Verhey[1], Philip Scheltens[2], Charlotte Teunissen[3], Sebastiaan Engelborghs[4,5,6], Kristel Sleegers[6,7], Giovanni Frisoni[8,9], Olivier Blin[10], Jill C. Richardson[11], Régis Bordet[12], Magda Tsolaki[13], Julius Popp[14,15], Gwendoline Peyratout[15], Pablo Martinez-Lage[16], Mikel Tainta[16], Alberto Lleó[17], Peter Johannsen[18], Yvonne Freund-Levi[19,20], Lutz Frölich[21], Rik Vandenberghe[22], Sarah Westwood[23], Valerija Dobricic[24], Frederik Barkhof[25,26], Cristina Legido-Quigley[27,28], Lars Bertram[24,29,30], Simon Lovestone[23], Johannes Streffer[5,31], Ulf Andreasson[32,33], Kaj Blennow[32,33], Henrik Zetterberg[32,33,34,35] and Pieter Jelle Visser[1,2]

Affiliations

[1]Alzheimer Centrum Limburg, Maastricht University, Maastricht, the Netherlands;

[2]Alzheimer Center, VU University Medical Center, Amsterdam, the Netherlands;

[3]Neurochemistry lab and Biobank, Department of Clinical Chemistry, VU University Medical Center, Amsterdam Neuroscience, the Netherlands;

[4]Department of Neurology and Memory Clinic, Hospital Network Antwerp (ZNA) Middelheim and Hoge Beuken, Antwerp, Belgium;

[5]Reference Center for Biological Markers of Dementia (BIODEM), Institute Born-Bunge, University of Antwerp, Antwerp, Belgium;

[6]Institute Born-Bunge, University of Antwerp, Belgium;

[7]Neurodegenerative Brain Diseases Group, Center for Molecular Neurology, VIB, Belgium;

[8]University of Geneva, Geneva, Switzerland;

[9]IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy;

[10]Mediterranean Institute of Cognitive Neuroscience, Aix Marseille University, Marseille, France;

[11]Neurosciences Therapeutic Area, GlaxoSmithKline R&D, Stevenage, U.K;

[12]University of Lille, Inserm, CHU Lille, France;

[13]1st Department of Neurology, AHEPA University Hospital, Makedonia, Thessaloniki, Greece;

[14]Geriatric Psychiatry, Department of Mental Health and Psychiatry, Geneva University Hospitals, Switzerland;

[15]Department of Psychiatry, University Hospital of Lausanne, Lausanne, Switzerland;

[16]Department of Neurology, Center for Research and Advanced Therapies, CITA-Alzheimer Foundation, San Sebastian, Spain;

[17]Hospital de la Santa Creu i Sant Pau, Barcelona, Spain;

[18]Danish Dementia Research Centre, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark;

[19]Department of Neurobiology, Caring Sciences and Society (NVS), Division of Clinical Geriatrics, Karolinska Institutet, and Department of Geriatric Medicine, Karolinska University Hospital Huddinge, Stockholm, Sweden;

[20]Department of Psychiatry Norrtälje Hospital Tiohundra, Norrtäije, Sweden;

[21]Department of Geriatric Psychiatry, Zentralinstitut für Seelische Gesundheit, University of Heidelberg, Mannheim, Germany;

[22]University Hospital Leuven, Leuven, Belgium;

[23]University of Oxford, Oxford, United Kingdom;

[24]Lübeck Interdisciplinary Platform for Genome Analytics, University of Lübeck, Germany;

[25]Department of Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, The Netherlands;

[26]Institutes of Neurology and healthcare engineering, University College London, UK;

[27]Steno Diabetes Center, Copenhagen, Denmark;

[28]King's College London, London, United Kingdom;

[29]School of Public Health, Imperial College London, UK;

[30]Department of Psychology, University of Oslo, Norway;

[31]Early Clinial Neurology, UCB Biopharma SPRL, Braine-l'Alleud,Belgium, formely Janssen R&D,

LLC. Beerse, Belgium at the time of study conduct;

[32]Clinical Neurochemistry Lab, Institute of Neuroscience and Physiology, Sahlgrenska University Hospital, Mölndal, Sweden;

[33]Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, University of Gothenburg, Mölndal, Sweden;

[34]Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK;

[35]UK Dementia Research Institute, London, UK.

*Corresponding author:

Isabelle Bos, MSc

Department of Psychiatry & Neuropsychology, School for Mental Health and Neuroscience, Alzheimer Center Limburg, Maastricht University

Universiteitssingel 40, Box 34, P.O. Box 616, 6200 MD Maastricht, the Netherlands.

E-mail: isabelle.bos@maastrichtuniversity.nl

Phone: +31 (0) 43 38 84113

ABSTRACT

INTRODUCTION: We investigated relations between amyloid-β (Aβ) status, *APOE*-ε4 and cognition, with cerebrospinal fluid (CSF) markers of Neurogranin (Ng), Neurofilament-light, (NFL), YKL-40 and Total tau (T-tau).

METHODS: We included 770 individuals with normal cognition, MCI and AD-type-dementia from the EMIF-AD Multimodal Biomarker Discovery study. We tested the association of Ng, NFL, YKL-40 and T-tau with Aβ status (Aβ- vs. Aβ+), clinical diagnosis *APOE* ε4 carriership, baseline cognition and change in cognition.

RESULTS: Ng and T-tau distinguished between Aβ+ from Aβ- individuals in each clinical group, while NFL and YKL-40 were associated with Aβ+ in non-demented individuals only. *APOE* ε4 carriership did not influence NFL, Ng and YKL-40 in Aβ+ individuals. NFL was the best predictor of cognitive decline in Aβ+ individuals across the cognitive spectrum.

DISCUSSION: Axonal degeneration, synaptic dysfunction, astroglial activation and altered tau metabolism are involved already in preclinical AD. NFL may be a useful prognostic marker.

KEYWORDS

Alzheimer's disease; amyloid-beta; neurofilament light; neurogranin; YKL-40; cognition; cerebrospinal fluid; *APOE*

1. Background

Biomarkers have become increasingly important for the diagnosis of Alzheimer's disease (AD) [1, 2], and are contributing to an improved understanding of the temporal pattern of AD pathophysiology. It has been shown that amyloid-beta (Aβ) deposition is one of the earliest detectable events in AD pathogenesis [3, 4], and that genetic risk for AD can be assessed by determining apolipoprotein E (*APOE)* ε4 genotype. However, other pathophysiological mechanisms underlying AD and their relation to inter-individual variation in cognitive trajectories, are less well understood. By relating Aβ, *APOE* genotype and cognition to cerebrospinal fluid (CSF) biomarkers for AD-related processes including axonal degeneration, synaptic dysfunction and astroglial activation in individuals across the clinical AD spectrum, we will likely learn more about the temporal ordering of these pathological mechanisms. This may translate into improved diagnostic and prognostic algorithms, which, in turn, should help to develop and evaluate more targeted disease-modifying treatments.

Besides Aβ, a number of proteins in CSF have been found to be associated with AD. Both phosphorylated (P-tau) and total tau (T-tau) are well-established biomarkers for AD and cognitive decline [5, 6]. High concentrations of neurofilament-light (NFL) have been associated with axonal degeneration to, predominantly, subcortical brain areas [7, 8] and YKL-40 (also known as chitinase 3-like protein 1) concentrations were found to reflect astrocytic activation, an inflammatory response to neurodegenerative processes [9]. Neurogranin (Ng) has been identified as a candidate AD marker reflecting synaptic degeneration and cognitive decline in the early stages of AD [10, 11]. While NFL, YKL-40 and Ng have evolved over the last years as promising AD biomarkers and have been strongly associated with neuronal injury markers [11-13], data regarding their relation to Aβ, *APOE* and cognition have been inconsistent or inconclusive [10, 12, 14-16].

Hence, to unravel how NFL, Ng and YKL-40 relate to AD pathology, genetic risk and disease severity, we aimed to investigate their relationships with Aβ, *APOE* ε4 carriership and cognition, in a large cohort consisting of individuals across the AD spectrum. To compare the relations regarding NFL, Ng and YKL-40 to those of an established neurodegenerative AD marker, we also examined the associations of T-tau with Aβ, *APOE* genotype and cognition.

2. Methods

2.1 Subjects

We selected 770 individuals from the EMIF-AD Multimodal Biomarker Discovery (EMIF-AD MBD) study; a cross-cohort study consisting of collated data and samples from 11 European cohorts [17]. The EMIF-AD MBD includes a total of 1221 individuals across the cognitive spectrum: normal cognition (NC), Mild Cognitive Impairment (MCI) and AD-type dementia. Individuals were selected from prospective cohort studies based on the availability of plasma, DNA and CSF samples and MRI scans. Exclusion criteria for the EMIF-AD MBD study were the presence of neurological, psychiatric or somatic disorders that could cause cognitive impairment [17]. Written informed consent was obtained from all participants before inclusion in the study. The medical ethics committee at each site approved the study (Supplemental Table 1).

For the current study we selected all participants from whom CSF samples were available for central analyses (n=770). Participants were included from three multicenter studies: DESCRIPA (n=29) [18], EDAR (n=197) [19] and IMI PharmaCog (n=146) [20], and four single center studies: Amsterdam (n=170) [21], Antwerp (n=148) [22], San Sebastian GAP (n=40) [23] and Lausanne (n=40) [24].

2.2 Clinical diagnosis and assessment

Normal cognition (NC) was defined as normal performance on neuropsychological assessment (within 1.5 SD of the average for age, gender and education). MCI was defined as having performance below 1.5 SD of the average on at least one neuropsychological test [25]. AD-type dementia was defined based on a clinical diagnosis, using the National Institute of Neurological and Communicative Disorders and Stroke – Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria [26].

The clinical assessment is described in a previous publication [17]. In short, clinical data were collected using local routine protocol at each site and thereafter harmonized and stored onto the EMIF-AD online data platform for pooled analyses. We used the Mini Mental State Examination (MMSE) [27] as our main cognitive outcome measure, which was available in 99% of the subjects at baseline and in 68% at follow-up. In general, baseline clinical assessment and CSF collection were conducted within a one year window. For a subgroup, the length of this time window was unknown (n=21) or longer than one year (n=2).

2.3 CSF analyses

Central CSF analyses were conducted at Gothenburg University, Sweden. NFL concentrations were measured using a commercial ELISA (NF-light® ELISA, Uman Diagnostics, Umeå, Sweden; [7]). Ng was measured using an in-house immunoassay for Ng [10]. YKL-40 was determined by a human chitinase-3 quantikine ELISA kit (R&D systems, Inc, Minneapolis, MN; [28]). $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ were measured using the V-PLEX Plus Aβ Peptide Panel 1 (6E10) Kit from Meso Scale Discovery (MSD, Rockville, MD). All analyses were performed according to the manufacturer's instructions by board-certified laboratory technicians who were blinded to clinical information. All measurement were performed on one occasion using

one batch of reagents, except for n=8 samples from the EDAR cohort that were analysed beforehand in the same laboratory, but in a different batch. For phosphorylated tau (P-tau) and total tau (T-tau), we used available measures from the local cohorts (P-tau n=630; T-tau n=621) derived in clinical laboratory practice using INNOTEST ELISAs (Fujirebio, Ghent, Belgium).

2.4 Genetic analyses

For the entire EMIF-AD BMD cohort *APOE* genotyping data from the local genetic analyses was available for n=1121 (91%) individuals. For central analyses, 805 DNA and 148 whole blood samples were transferred to Lübeck University, Germany. From the blood samples, DNA was extracted using QIAamp® DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) resulting in 953 DNA samples, of which 926 passed quality control. All samples were subjected to genome-wide SNP genotyping using the Infinium Global Screening Array (GSA) with Shared Custom Content (Illumina Inc.). From these genome-wide data, *APOE* genotypes were determined either directly (rs7412) or by imputation (rs429358) in all 926 samples. For 80 samples for which no local *APOE* genotype was available, and for 45 mismatches between locally and GSA derived genotypes (4.8%), *APOE* genotype was determined using TaqMan assays (ThermoFisher Scientific, Foster City, CA) on a QuantStudio-12K-Flex system in 384-well format. We classified individuals as *APOE* ε4 carriers (ε4+) or non-carriers (ε4-) according to their genotype status at rs429358 (C-allele = ε4).

2.5 Biomarker classifications

Aβ status was defined by the CSF $A\beta_{42/40}$ ratio, using a cut-off of <0.063 to determine abnormality. This cut-off was defined using mixture model analyses in the current dataset [29, 30], showing a clear binomial distribution (Supplemental Figure 1). Abnormality based on this cut-off showed a high concordance rate with abnormality based on the local $A\beta_{42}$ measures

and cut-offs (82%). For the analyses regarding the influence of NFL, Ng and YKL-40 on cognition, a median-split was used to divide the sample (Cut-off values: NFL: 869 pg/ml; Ng: 103 pg/ml; YKL-40: 163 ng/ml) as there are no well-established cut-offs or approaches yet to define abnormality and the use of tertiles or quartiles to divide the data would limit statistical power.". Dichotomous T-tau values (normal vs. abnormal) was available in n=762 individuals and was determined using local cut-off points (Supplemental Table 2).

2.6 Statistical analyses

Baseline characteristics were compared by Aβ status and diagnostic group using Chi-square for categorical variables and general linear mixed (GLM) models with study as a random effect for continuous variables. We also tested whether the influence of Aβ on NFL, Ng and YKL-40 was different across diagnostic groups and age, by examining the diagnostic group by Aβ, and age by Aβ interactions. Prior to the comparisons, $A\beta_{42}$, NFL, Ng, YKL-40, P-tau and T-tau values were log-transformed to approximate a normal distribution. Spearman's correlations were used to assess the correlations between biomarker values. GLM models with random intercepts and slopes by study were used to examine the influence of Aβ status and low/high or normal/abnormal biomarker levels on MMSE performance and decline over time, adjusted for age, gender, years of education and baseline diagnosis. Lastly, we tested the independent influence of all markers on cognitive decline by adding all dichotomous markers (high/low or normal/abnormal) in one GLM model with MMSE scores over time as outcome measure, stratified by Aβ status. Missing values for *APOE* ε4 status (n=12) and years of education (n=105) were imputed using regression analyses within study, based on significant predictors (i.e. age, gender, MMSE, cognitive scores) for these variables. All analyses were repeated after exclusion of individuals with a long or unknown interval between clinical assessment and CSF collection (n=23). Statistical analyses were performed using R Statistical Software (version

3.3.3) and SPSS (version 24). We used two-sided $p<0.05$ to define statistical significance. Due to the exploratory nature of the study we did not adjust for multiple comparisons.

3. Results

We assessed 770 individuals who were on average 69.3 (SD 8.3) years old and had an average of 10.9 (SD 3.9) years of education. Three hundred ninety-nine (52%) were female. Clinical follow-up data was available for 557 (73%) individuals, with an average follow-up length of 2.3 (SD 1.3) years. At baseline 140 (18%) individuals were considered cognitively normal (CN), 450 (58%) were diagnosed as having mild cognitive impairment (MCI) and 180 (23%) were clinically diagnosed as having AD-type dementia. Despite a clinical diagnosis of AD-type dementia, 23 (13%) individuals did not show evidence of amyloid pathology.

3.1 Demographics and biomarker values

Table 1 shows the baseline characteristics and biomarker values per diagnostic group, stratified by Aβ status. As expected, in the whole sample, Aβ+ individuals were older, more frequently *APOE*-ε4 carrier and had lower MMSE scores compared to Aβ- individuals. When stratified by baseline diagnosis, we found that Aβ+ individuals were older compared to the Aβ- individuals in the CN and MCI groups, but not in the AD-type dementia group. Only in MCI we found a difference in MMSE score between groups by Aβ status. Other comparisons are shown in Table 1.

3.2 NFL, Ng, YKL-40 and T-tau by Aβ status and baseline diagnosis

Comparisons by Aβ status and baseline diagnoses of NFL, Ng, YKL-40 and T-tau concentrations are shown in Table 1. Figure 1 shows the comparisons by Aβ status within the diagnostic groups. When comparing by Aβ status, NFL and YKL-40 values were differentially

increased in Aβ+ CN and MCI individuals, while in the dementia stage NFL and YKL-40 levels were elevated regardless of Aβ status. T-tau and Ng values were stably increased in Aβ+ individuals across the cognitive spectrum. For NFL we found that the influence of Aβ on NFL was different across diagnoses (interaction Aβ*diagnosis p=0.027). NFL concentrations increased in Aβ- individuals with advancing clinical stage, while they were stable in the Aβ+ CN and MCI groups but increased further in the Aβ+ AD-type dementia group (Figure 1). The influence of Aβ on YKL-40 levels was similar as for NFL (interaction Aβ*diagnosis p=0.001). For Ng and T-tau we found that influence of Aβ was similar across diagnoses (interaction Aβ*diagnosis T-tau: p=0.771;Ng: p=0.580). Aβ+ did have a stronger effect on Ng and T-tau concentrations in younger individuals than in older individuals (interaction Aβ*age Ng: p=0.006; T-tau: p<0.001), while there was no age effect for NFL and YKL-40 (data not shown).

3.3 APOE ε4 carriership

In Aβ+ individuals, no effect was found of *APOE* ε4 carriership on NFL, Ng and YKL-40 levels, regardless of clinical diagnosis (Table 2). In Aβ- individuals, *APOE* ε4 carriership was associated with lower levels of NFL in the total group and in individuals with MCI, as well as with lower Ng levels in the MCI and AD-type dementia groups, but with higher Ng levels in the total group (Table 2). We found no influence of *APOE* ε4 carriership on YKL-40 and T-tau levels when comparing within Aβ status, stratified by diagnosis. However, compared to the CN Aβ- *APOE* ε4 non-carriers, T-tau and YKL-40 levels were elevated in Aβ+ individuals regardless of clinical diagnosis (Table 2).

3.4 Correlations

The Aβ isoforms were highly positively correlated and a more abnormal $A\beta_{42/40}$ ratio was correlated with higher NFL, Ng and YKL-40 levels. P-tau and t-tau were highly correlated, and were both associated with all three emerging biomarkers (Supplemental Figure 2).

3.5 Baseline cognition and change in cognition over time

Cross-sectional analyses showed that in Aβ+ individuals, high NFL, Ng and T-tau levels were associated with lower MMSE scores in the total group (Table 3, Figure 2). When stratifying by diagnostic group within the Aβ+ individuals, high NFL levels were associated with low MMSE scores in the MCI and AD-type dementia groups, and high T-tau levels with low MMSE scores in the MCI group (Table 3). In Aβ- individuals, high NFL levels were associated with lower MMSE scores in the total group, and high T-tau levels with lower scores in the AD-type dementia group. In addition, high Ng levels were associated with higher MMSE scores in the AD-type dementia group in Aβ- individuals.

Longitudinal analyses showed that in Aβ+ individuals, high baseline levels of NFL and T-tau were associated with an increased rate of cognitive decline in the total sample. High baseline levels of NFL and Ng were also associated with increased rate of decline in the AD-type dementia group. In Aβ- individuals, high baseline levels of NFL, YKL-40 and T-tau were associated with an increased rate of cognitive decline in the total group, as well as in the MCI and AD-type dementia groups (Table 3). In Aβ- individuals, high Ng levels were associated with a decreased rate of decline in the MCI group, but with an increased rate of decline in the AD-type dementia group (Table 3).

Next, we combined NFL, YKL-40, Ng, and T-tau in the longitudinal analyses and stratified by baseline diagnosis (Table 4). In CN Aβ+ individuals, only high baseline NFL levels predicted

decline. In Aβ+ individuals with MCI, increased baseline NFL and T-tau and decreased Ng levels independently predicted cognitive decline. In Aβ+ individuals with AD-type dementia, increased baseline NFL and Ng levels predicted decline. Among Aβ- individuals, increased baseline NFL and tau levels predicted decline only in individuals with MCI (Table 4).

When repeating all analyses without the individuals for whom the interval between CSF collection and cognition was longer than one year or unknown (n=23), results remained similar. Exclusion of an individual with very high Ng concentrations also yielded similar results. In addition, outcomes were also similar when using P-tau instead of T-tau in the analyses regarding APOE ε4 carriership and cognition.

4. Discussion

We investigated the relations between Aβ status, *APOE* ε4 carriership and cognition, with CSF concentrations of NFL, Ng, YKL-40 and T-tau, in a large cohort of individuals across the clinical AD spectrum. The main findings were: (1) CSF NFL, Ng, YKL-40 and T-tau levels were associated with Aβ already in the preclinical stage; (2) Aβ- *APOE* ε4 carriers with MCI or AD-type dementia had lower concentrations of NFL and Ng compared to non-carriers; (3) High baseline NFL levels predicted cognitive decline in Aβ+ individuals with normal cognition, MCI and AD-type dementia, independent of the other markers.

NFL, Ng, YKL-40 and T-tau concentrations were all associated with Aβ+. In Aβ+ individuals, NFL levels were higher in the dementia stage compared to the MCI stage, whereas Ng and YKL-40 levels stayed relatively stable over time. Yet in Aβ- individuals, we found an increase of both NFL and YKL-40 levels in MCI individuals compared to CN individuals, while Ng levels in Aβ- individuals remained low with increasing disease severity. T-tau levels increased

with disease severity regardless of Aβ status, albeit the rate of increase was faster in Aβ+ individuals. These findings confirm that synaptic dysfunction – as measured by Ng – plays an important role in AD pathophysiology in all clinical stages [31, 32]. In addition, our data verifies that axonal degeneration and neuroinflammation - as respectively measured by NFL and YKL-40 – are less specific to AD [9, 33], but their temporal pattern across the clinical stages is AD specific: in AD, NFL and YKL-40 levels are already increased in the preclinical stage, while in Aβ- individuals concentrations merely start to increase from the MCI stage onwards. Our findings regarding T-tau levels, confirm the association of altered neuronal tau metabolism with Aβ pathology [6, 34], and support the notion this process also occurs in Aβ- individuals, although to a lesser extent [35]. Together these results provide novel insights into the temporal pattern of AD pathophysiology, which should be validated by longitudinal biomarker studies.

The APOE genotype did not influence NFL, Ng, YKL-40 and T-tau levels in Aβ+ individuals in all clinical stages, suggesting that these markers reflect a generic reaction to amyloid aggregation regardless of *APOE* genotype. In Aβ- individuals, *APOE* ε4 carriers with MCI or AD-type dementia had lower NFL and Ng levels compared to non-carriers. This suggests that the Aβ- *APOE* ε4 non-carriers with MCI or AD-type dementia might have other pathologies not related to Aβ and *APOE* ε4 carriership that are causing cognitive impairment, axonal degeneration, and to a lesser extent also synaptic dysfunction. Regarding T-tau and YKL-40 levels, we found similar concentrations in *APOE* ε4 carriers and non-carriers, which is in line with previous studies [36-38], but in contrast with a previous study in which a modest association of *APOE* ε4 carriership on YKL-40 levels was found in individuals with MCI due to AD [39]. Besides the inconsistency with the latter study, possibly due to heterogeneity in

sample sizes or biomarker classifications, our results confirm that YKL-40 concentrations are independent of *APOE* ε4 carriership.

Higher levels of NFL and T-tau were associated with a lower cognitive performance and an increased rate of decline regardless of Aβ status. As both NFL and T-tau are markers of axonal degeneration [5, 12], these findings imply that axonal loss may be an important driver of cognitive decline in both Aβ+ and Aβ- individuals [33, 40]. Concerning Ng, we found that only in the dementia stage, higher concentrations were associated with a faster rate of decline, regardless of Aβ. This is congruent with previous CSF biomarker studies suggesting that Ng might be strongly associated with cognition, irrespective of amyloid plaque pathology [40-42]. However, Ng changes have also been associated with cognitive decline in preclinical AD [11], a finding we could not confirm with our analyses possibly due to a lower sensitivity of the cognitive outcome measure we used (i.e. MMSE) or because we used a median-split instead of tertiles to define low and high Ng levels. Posthoc, we explored the influence of the cognitive outcome measure by repeating the analyses in a subgroup (n=615) with a pooled standardized memory score [17]. These posthoc analyses showed that high Ng levels tended to be associated with a faster decline in memory performance in CN Aβ+, but not in CN Aβ- individuals (data not shown). The negative impact of high YKL-40 levels on cognition seems to only relate to Aβ- individuals or the influence is masked by Aβ pathology in Aβ+ individuals. These findings suggest that YKL-40 may be a prognostic marker for individuals with MCI but without evidence of Aβ pathology, for instance those with Suspected Non-Alzheimer's Disease Pathophysiology (SNAP) [43]. When all markers were combined in one model we found that NFL, and from the MCI stage onwards also T-tau, were independent predictors of cognitive decline in Aβ+ individuals. Remarkably high Ng levels were associated with a slower rate of decline in Aβ+ individuals with MCI and a faster rate of decline in Aβ+ individuals with AD-

type dementia. Although a similar finding was described in a previous study [42], it remains uncertain what the underlying mechanism is. Possibly, Ng is not a direct contributor to cognitive decline in the pre-dementia stages or the relation between Ng and cognition is again dependent on the cognitive outcome measure used (global cognition vs. memory).

This study has several limitations. First, data was collected at different centers using routine local protocols. However, the CSF samples were analyzed centrally for most outcome measures – $A\beta_{38}$, $A\beta_{40}$, $A\beta_{42}$, NFL, Ng and YKL-40 - and clinical data was harmonized using validated methods like standardization and dichotomization. Second, our AD-type dementia group contained Aβ- individuals; a consequence of using a clinical diagnosis for classification, instead of a biomarker-based diagnosis. Although this makes our demented group more heterogeneous, it does reflect current clinical practice and is in line with earlier research showing that ~20% of individuals with AD dementia are Aβ- [44]. Third, our clinical follow-up may have been too short to obtain an accurate view of cognitive trajectories over time. And lastly, we chose the MMSE to assess cognition as this data was available in nearly all individuals, but it might not be sensitive enough to detect subtle cognitive decline and decline in specific cognitive domains. Future studies with longer follow-up and employing other cognitive measures should therefore validate our results regarding cognitive decline.

In conclusion, we found that NFL, Ng and YKL-40 were associated with Aβ pathology, showing that axonal degeneration, synaptic dysfunction and neuroinflammation are all to some extent involved in AD pathophysiology. Furthermore, we found that NFL is a generic prognostic marker which is elevated early in AD, and has a profound influence on cognition. Ng is a useful AD marker as it is closely related to Aβ and tau in all cognitive stages and is associated with cognition. YKL-40 has an influence on cognitive decline in absence of Aβ, and

thereby may be of value to increase the accuracy of the prognosis of individuals with SNAP. Lastly, our data identifies NFL as the strongest predictor of cognitive decline in Aβ+ individuals across the cognitive stages. Altogether, our findings improve prognostic accuracy and increase our knowledge of biomarker changes in relation to disease evolution.

Authors' contributions

Study concept and design: IB, SV, HZ & PJV. Acquisition and/or interpretation of data or samples: all authors. Statistical analysis and drafting the manuscript: IB, SV, & PJV. Critical revision of final draft of manuscript: all authors.

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Competing interests

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Figure Legends

Figure 1. CSF NFL, Ng, YKL-40 and T-tau levels by diagnostic groups and Aβ status

Boxplots (displaying first quartile, median and third quartile) and scatterplots of CSF neurogranin (Ng), neurofilament (NFL) and YKL-40 by diagnostic groups and by Aβ status (Aβ-: green; Aβ+: orange). *p<0.05, **p<0.01, ***p<0.001 comparisons by Aβ status within diagnostic group. Figure A shows log transformed NFL concentrations, Figure B shows log transformed Ng concentrations and Figure C shows log transformed YKL-40 concentrations. Figure D shows log transformed T-tau concentrations.

Figure 2. Influence of CSF NFL, Ng, YKL-40 and T-tau on cognition in the total group.

The graphs show mean scores and 95% confidence intervals of cognitive performance over time for high (red) and low (blue) CSF biomarker levels and by Aβ status (dashed lines: Aβ-; solid lines: Aβ+). *p<0.05 comparisons within Aβ group, **p<0.01 comparisons within Aβ group, ***p<0.001 comparisons within Aβ group. Figure A shows the influence of NFL levels. Figure B shows the influence of Ng levels. Figure C shows the influence of YKL-40 levels. Figure D shows the influence of T-tau levels.

Figure 3. Schematic overview of associations between NFL, Ng and YKL-40 with APOE ε4 positivity and cognition by diagnostic group and Aβ status

This figure shows the various associations examined in this study. In the top panel the associations in cognitively normal are visualized. In the middle panel the associations in individuals with MCI are visualized and in the bottom panel the association in individuals with AD-type dementia. The green arrows represent association in Aβ- individuals, the orange arrow represent association in Aβ+ individuals. Negative association are visualized with a minus (-) and positive association with a plus (+).

Table 1. Baseline characteristics and CSF biomarker values across the diagnostic groups and by Aβ status

	CN		MCI		AD-type dementia	
	Aβ- n=95 (A)	Aβ+ n=45 (B)	Aβ- n=187 (C)	Aβ+ n=263 (D)	Aβ- n=23 (E)	Aβ+ n=157 (F)
Age	62.7 ± 7.3^{B,C,D,E,F}	69.5 ± 8.1^{A,E}	68.6 ± 8.2^{A,D,E}	71.4 ± 7.1^{A,C,F}	74.2 ± 7.9^{A,B,C,F}	69.8 ± 8.8^{A,D,E}
Female, n	49 (52)	23 (51)	89 (48)	145 (55)	8 (34)	85 (54)
Education in years	12.6 ± 3.5^{C,D,E,F}	12.2 ± 3.9^{C,D,E,F}	10.4 ± 3.8^{A,B,E}	11.0 ± 3.6^{A,B,E}	8.6 ± 4.7^{A,B,C,D,F}	10.6 ± 3.6^{A,B,E}
APOE-ε4 carrier, n	28 (30)^{B,C,D,F}	27 (60)^{A,C,E}	38 (20)^{A,B,D,F}	175 (67)^{A,C,E}	5 (22)^{B,D,F}	104 (66)^{A,C,E}
MMSE	28.7 ± 1.2^{C,D,E,F}	28.7 ± 1.3^{C,D,E,F}	26.8 ± 2.4^{A,B,D,E,F}	25.8 ± 2.6^{A,B,C,E,F}	22.4 ± 4.5^{A,B,C,D}	21.3 ± 4.8^{A,B,C,D}
$Aβ_{38}$, pg/ml	2245.7 ± 834.3	2405.5 ± 670.0^{F}	2247.3 ± 948.2^{F}	2160.2 ± 858.6^{F}	2447.4 ± 1248.2	2139.6 ± 834.8^{B,C,D}
$Aβ_{40}$, pg/ml	5217.7 ± 1709.4	5585.8 ± 1470.9^{F}	5190.4 ± 1970.7^{F}	4939.9 ± 1824.2^{F}	5556.8 ± 2269.6	5078.1 ± 1801.5^{B,C,D}
$Aβ_{42}$, pg/ml	466.2 ± 182.8^{B,D,F}	254.4 ± 75.0^{A,C,E,F}	467.2 ± 218.2^{B,D,F}	211.6 ± 88.8^{A,C,E,F}	461.4 ± 217.6^{B,D,F}	215.9 ± 89.4^{A,B,C,D,E}
$Aβ_{42/40}$ ratio	0.089 ± 0.01^{B,D,E,F}	0.045 ± 0.01^{A,C,D,E}	0.089 ± 0.02^{B,D,F}	0.04 ± 0.01^{A,C,E}	0.08 ± 0.01^{B,D,F}	0.04 ± 0.01^{A,C,E}
P-tau, pg/ml$^{\#}$	38.7 ± 12.4^{B,C,D,F}	61.5 ± 27.3^{A,C,D,F}	48.2 ± 18.6^{A,B,D,F}	80.3 ± 32.8^{A,B,C,E}	41.5 ± 17.4^{D,F}	86.2 ± 41.1^{A,B,C,E}
T-tau, pg/ml$^{\#}$	197.3 ± 72.5^{B,C,D,F}	405.2 ± 330.0^{A,C,D,F}	280.4 ± 134.2^{A,B,D,F}	572.3 ± 315.9^{A,B,C,E}	225.3 ± 82.7^{D,F}	708.0 ± 445.0^{A,B,C,E}
NFL, pg/ml	627.4 ± 293.3^{B,C,D,E,F}	983.13 ± 678.4^{A,E,F}	1031.2 ± 919.1^{A,D,E,F}	1242.3 ± 2556.1^{A,C,F}	1931.9 ± 1934.8^{A,C}	1742.2 ± 2893.2^{A,B,C,D}
Ng, pg/ml	110.8 ± 224^{B,D,F}	152.6 ± 149.6^{A,C}	99.2 ± 102.9^{B,D,F}	175.5 ± 217.8^{A,C,E}	118.3 ± 136.0^{D,F}	155.2 ± 121.4^{A,C,E}
YKL-40, ng/ml	127.0 ± 45.4^{B,C,D,E,F}	175.1 ± 63.6^{A}	162.2 ± 65.2^{A,D,F}	183.4 ± 60.5^{A,C}	184.2 ± 64.6^{A}	193.6 ± 68.7^{A,C}

Results are mean ± SD or number (%). Biomarker comparisons were done with the log transformed values for $Aβ_{42}$, NFL, Ng, YKL-40, p-tau and t-tau, and adjusted for age, gender, *APOE*-ε4 carrier status and with study as a random effect. $^{\#}$P-tau and t-tau values were analyzed locally and available in a subgroup p-tau: CN n=103, MCI n=403, AD n=124; t-tau: CN n=103, MCI n=399, AD n=119. ^{A} p<0.05 compared to CN Aβ-, ^{B} p<0.05 compared to CN Aβ+, ^{C} p<0.05 compared to MCI Aβ-, ^{D} p<0.05 compared to MCI Aβ+, ^{E} p<0.05 compared to AD dementia Aβ-, ^{F} p<0.05 compared to AD dementia Aβ+. Abbreviations: Aβ= amyloid-beta; AD = Alzheimer's Disease; *APOE* = Apolipoprotein E; CN = cognitively normal; MCI = Mild Cognitive Impairment; NFL = neurofilament light; Ng = neurogranin; P-tau = phosphorylated tau; T-tau = total tau.

Table 2. Comparisons of CSF NFL, Ng, YKL-40 and T-tau concentrations by APOE ε4 status within Aβ group

		Aβ-			Aβ+		
Biomarker	Group	number (ε4-/ ε4+)	ε4-	ε4+	number (ε4-/ ε4+)	ε4-	ε4+
NFL, pg/ml	All	233/70	1042.5 ± 69.1	728.7 ± 50.1*	159/299	1460.5 ± 246.7^	1349.7 ± 129.5^
	CN	67/28	**627.1 ± 33.5**	628.2 ± 64.2	18/27	1044.2 ± 117.5	942.4 ± 150.6
	MCI	148/38	1091.9 ± 81.7^	795.0 ± 77.0*	88/168	1509.8 ± 441.7^	1102.2 ± 76.1^
	AD-type dementia	18/4	2183.1 ± 485.7^	801.7 ± 123.8	53/104	1519.9 ± 98.7^	1855.4 ± 345.0^
Ng, pg/ml	All	202/63	101.7 ± 6.8	111.7 ± 32.5**	149/292	167.3 ± 11.8^	166.0 ± 11.8^
	CN	54/24	**91.3 ± 11.2**	154.7 ± 79.3	15/27	194.4 ± 57.5^	129.3 ± 16.2
	MCI	132/35	101.3 ± 8.2	91.4 ± 22.1*	81/169	169.1 ± 15.9^	178.5 ± 18.9^
	AD-type dementia	16/4	140.1 ± 36.1	31.1 ± 8.1*	53/96	156.8 ± 16.0^	154.3 ± 12.7^
YKL-40, ng/ml	All	234/71	156.0 ± 4.2	142.6 ± 6.9	158/305	192.9 ± 4.8^	182.5 ± 3.7^
	CN	67/28	**123.3 ± 4.8**	136.0 ± 11.0	18/27	180.8 ± 16.8^	171.3 ± 11.4^
	MCI	149/38	165.4 ± 5.5^	149.6 ± 9.4^	87/174	187.6 ± 5.9^	181.3 ± 4.8^
	AD-type dementia	18/5	200.2 ± 14.9^	126.8 ± 11.1	53/104	205.7 ± 8.9^	187.5 ± 6.9^
T-tau, pg/ml	All	170/47	266.2 ± 10.1	221.3 ± 15.1	125/240	627.7 ± 39.9^	576.8 ± 20.5^
	CN	43/15	**198.3 ± 10.7**	194.6 ± 21.1	14/21	455.8 ± 131.8^	371.5 ± 33.9^
	MCI	119/29	292.2 ± 12.6^	232.0 ± 20.3	78/150	578.5 ± 44.2^	569.1 ± 22.1^
	AD-type dementia	8/3	182.5 ± 36.5	332.3 ± 122.1	33/69	816.8 ± 87.3^	656.0 ± 43.2^

Results are mean ± SE. Comparisons were conducted between log-transformed biomarker concentrations and adjusted for age, gender and study. *p<0.05, **p<0.01, ***p<0.001 as compared to the ε4- within the Aβ group. ^p<0.05 compared to the CN Aβ- ε4- group (in bold). Abbreviations: Aβ= amyloid-beta; AD = Alzheimer's Disease; CN = cognitively normal; MCI = Mild Cognitive Impairment; NFL = neurofilament light; Ng = neurogranin.

Table 3. Influence of CSF NFL, Ng, YKL-40 and T-tau on cognitive performance and decline by Aβ status

		Aβ-			Aβ+		
Biomarker	Group	number (low/high)[#]	Baseline difference	Slope difference	number (low/high)[#]	Baseline difference	Slope difference
NFL	All	194/109	-0.98 ± 0.44*	-0.40 ± 0.13**	182/276	-1.89 ± 0.34***	-0.39 ± 0.10***
	CN	74/21	0.14 ± 0.78	0.40 ± 0.27	28/17	-0.36 ± 1.03	-0.40 ± 0.40
	MCI	112/74	-0.86 ± 0.45	-0.51 ± 0.14***	122/134	-0.72 ± 0.36*	0.04 ± 0.17
	AD-type dementia	8/14	-2.53 ± 1.39	-0.33 ± 0.71*	32/125	-1.71 ± 0.68*	-0.60 ± 0.25*
Ng	All	171/94	0.51 ± 0.45	0.21 ± 0.10	182/259	-0.58 ± 0.34*	-0.15 ± 0.11
	CN	52/26	0.45 ± 0.80	0.17 ± 0.25	17/25	0.49 ± 1.08	-0.29 ± 0.37
	MCI	108/59	0.10 ± 0.48	0.25 ± 0.12*	109/141	-0.52 ± 0.36	-0.24 ± 0.16
	AD-type dementia	11/9	4.90 ± 1.49**	-2.48 ± 0.74**	56/93	0.01 ± 0.62	-0.76 ± 0.22**
YKL-40	All	198/107	-0.45 ± 0.42	-0.44 ± 0.13**	186/277	0.07 ± 0.34	0.01 ± 0.10
	CN	74/21	-0.36 ± 0.82	0.29 ± 0.20	20/25	-0.32 ± 1.00	-0.32 ± 0.40
	MCI	113/74	0.07 ± 0.43	-0.60 ± 0.11***	111/150	0.18 ± 0.35	0.15 ± 0.16
	AD-type dementia	11/12	-2.12 ± 1.36	-1.40 ± 0.59*	55/102	0.79 ± 0.60	0.22 ± 0.23
T-tau	All	236/66	-0.67 ± 0.49	-0.77 ± 0.14***	106/355	-1.64 ± 0.37***	-0.38 ± 0.12**
	CN	85/10	0.71 ± 1.01	0.02 ± 0.36	23/21	-0.26 ± 1.01	0.01 ± 0.41
	MCI	141/43	-0.51 ± 0.51	-0.79 ± 0.12***	60/201	-0.87 ± 0.40*	-0.18 ± 0.21
	AD-type dementia	10/13	-2.96 ± 1.37*	-0.96 ± 0.56*	23/133	-0.41 ± 0.81	-0.41 ± 0.31

Baseline differences in MMSE scores are mean difference ± standard error between low and high NFL, Ng and YKL-40 groups defined by median-split. Slopes are linear mixed model coefficient indicating annual decline ± standard error, relative to group with low biomarker level with MMSE score as outcome. *p<0.05, **p<0.01, ***p<0.001 compared to group with low biomarker levels, adjusted for age, gender, education level and study. Comparisons in the total sample were also adjusted for baseline diagnosis. [#]Number with low and high biomarker levels at baseline, for t-tau number with normal and abnormal t-tau levels at baseline.

Table 4. Independent influence of biomarkers on cognitive decline across the diagnostic groups

		Aβ-		Aβ+	
		β	p-value	β	p-value
CN	High NFL	0.20 ± 0.31	0.508	-1.19 ± 0.39	**0.004**
	High Ng	0.27 ± 0.21	0.216	-0.54 ± 0.35	0.134
	High YKL-40	-0.09 ± 0.26	0.741	0.28 ± 0.31	0.367
	High T-tau	-0.10 ± 0.30	0.737	0.48 ± 0.38	0.219
MCI	High NFL	-0.30 ± 0.15	**0.045**	-0.74 ± 0.26	**0.001**
	High Ng	0.28 ± 0.14	0.060	0.46 ± 0.16	**0.005**
	High YKL-40	-0.19 ± 0.16	0.242	0.12 ± 0.15	0.430
	High T-tau	-0.43 ± 0.18	**0.017**	-0.58 ± 0.22	**0.009**
AD-type dementia	High NFL	2.83 ± 2.77	0.857	-0.91 ± 0.35	**0.009**
	High Ng	0.42 ± 2.76	0.993	-0.64 ± 0.27	**0.021**
	High YKL-40	-9.12 ± 3.77	0.939	0.32 ± 0.31	0.315
	High T-tau	4.48 ± 2.65	0.971	-0.74 ± 0.43	0.084

Numbers are linear mixed model coefficients ± standard error with MMSE scores over time as dependent variable adjusted for age, gender and years of education. All CSF variables were entered at the same step. NFL, Ng and YKL-40 were dichotomized was based on median-split, T-tau based on the local cut-off for abnormality. Abbreviations: Aβ = amyloid-beta, CN = cognitively normal, MCI = Mild Cognitive Impairment, NFL = Neurofilament light, Ng = neurogranin, T-tau = Total tau.