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Serial CSF sampling in Alzheimer's disease: specific versus non-specific markers

Maartje I. Kester[a,*], Peter G. Scheffer[b], Marleen J. Koel-Simmelink[b], Harry Twaalfhoven[b], Nicolaas A. Verwey[a,b], Robert Veerhuis[b], Jos W. Twisk[c], Femke H. Bouwman[d], Marinus A. Blankenstein[b], Philip Scheltens[a], Charlotte Teunissen[b], Wiesje M. van der Flier[a,c]

[a] *Alzheimer Center and Department of Neurology, VU University Medical Center, Amsterdam, The Netherlands*

[b] *Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands*

[c] *Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands*

[d] *Department of Neurology, Catharina Hospital, Eindhoven, The Netherlands*

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Abstract

In this longitudinal study we investigated change over time in cerebrospinal fluid (CSF) levels of amyloid-beta 40 and 42 (Aβ40 and Aβ42), total tau (tau), tau phosphorylated at threonine 181 (ptau-181), isoprostane, neurofilaments heavy (NfH) and light (NfL). Twenty-four nondemented subjects, 62 mild cognitive impairment (MCI) and 68 Alzheimer's disease (AD) patients underwent 2 lumbar punctures, with minimum interval of 6, and a mean $\pm$ SD of 24 $\pm$ 13 months. Linear mixed models were used to assess change over time. Amyloid-beta 42, tau, and tau phosphorylated at threonine 181, differentiated between diagnosis groups ($p < 0.05$), whereas isoprostane, neurofilaments heavy, and NfL did not. In contrast, effects of follow-up time were only found for nonspecific CSF biomarkers: levels of NfL decreased, and levels of isoprostane, amyloid-beta 40, and tau increased over time ($p < 0.05$). Isoprostane showed the largest increase. In addition, increase in isoprostane was associated with progression of mild cognitive impairment to AD, and with cognitive decline as reflected by change in Mini Mental State Examination (MMSE). Contrary to AD-specific markers, nonspecific CSF biomarkers, most notably isoprostane, showed change over time. These markers could potentially be used to monitor disease progression in AD.
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Keywords: Alzheimer's disease; CSF biomarkers; Longitudinal

1. Introduction

Major efforts are under way to investigate therapeutic strategies that have the potential to slow progression of Alzheimer's disease (AD). To evaluate the effect of these interventions, biological markers are needed that reflect progression of AD pathology.

The major pathological hallmarks of AD are senile plaques, containing beta-amyloid and neurofibrillary tangles with microtubule-associated tau protein (McKhann et al., 1984). Cerebrospinal fluid (CSF) biomarkers amyloid-beta 1–42 (Aβ42), total tau (tau), and tau phosphorylated at threonine 181 (ptau-181) reflect the neuropathology of AD and are useful as diagnostic markers for AD (Blennow and Hampel, 2003). Several studies evaluated whether these markers could also be used as markers to monitor disease progression, but until now these biomarkers showed little effect in longitudinal settings (Blennow et al., 2007; Bouwman et al., 2007; Buchhave et al., 2009; Li et al., 2007; Mollenhauer et al., 2005; Zhou et al., 2009).

The specific biomarkers, amyloid-beta 42 (Aβ42), total tau (tau) and ptau-181, seem less suitable as biomarkers for monitoring of disease progression. Amyloid plaque deposi-

* Corresponding author at: Alzheimer Center, Department of Neurology, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands. Tel.: +31 20 4440742; fax: +31 20 4440715.

E-mail address: m.kester@vumc.nl (M.I. Kester).

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tion and tau tangle formation are early processes in AD, that may show little or no change later on (Jack et al., 2010). Other, more general and thus less specific disease processes are increasingly considered to play a major role in advanced stages of the disease (Jack et al., 2010). Oxidative stress damage is a process of neurotoxicity due to free radical-mediated damage to cellular membranes, which probably also occurs in advanced stages of AD (Quinn et al., 2004). Isoprostane, an oxidative stress marker, therefore, could be a useful marker to monitor AD. In fact, a few small studies have shown increase over time in isoprostane (de Leon et al., 2007; Montine et al., 2005; Quinn et al., 2004). Neurofilaments are released from damaged neurons. CSF levels of neurofilaments have been shown to reflect the degree of neuronal degeneration and axonal loss in several neurological diseases (de Jong et al., 2007; Petzold, 2005). Few cross-sectional studies have shown increased levels of neurofilaments in AD (Norgren et al., 2003; Pijnenburg et al., 2007), and possibly changes in the levels of neurofilaments also reflect progression of the disease. Furthermore, we hypothesized that CSF amyloid-beta n-40 (Aβ40) could be a biomarker for disease progression, because Aβ40 has been associated with solid, less diffuse, types of amyloid plaques, that generally develop in later stages of AD (Iwatsubo et al., 1994; Kumar-Singh, 2008).

We aimed to assess longitudinal effects of CSF biomarkers, in order to identify biomarkers that are useful to monitor disease progression. Our panel of 7 CSF biomarkers included Aβ42, tau and ptau-181, and several less specific CSF biomarkers, isoprostane, neurofilaments heavy (NfH), neurofilaments light (NfL), and Aβ40. We evaluated changes in CSF biomarker levels over time, and associations of change in CSF biomarker levels with change in Mini Mental State Examination (MMSE), in a large cohort of AD and mild cognitive impairment (MCI) patients and nondemented subjects.

2. Methods

2.1. Patients

We included patients with AD ($n = 68$), MCI ($n = 62$), and nondemented subjects ($n = 24$) with CSF at 2 time points. At baseline all patients underwent standard dementia screening including physical and neurological examination, laboratory tests, electroencephalogram (EEG), and magnetic resonance imaging (MRI). Cognitive screening included an MMSE, but usually involved comprehensive neuropsychological testing. The diagnosis of probable AD was made according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984). The diagnosis of MCI was made according to Petersen's criteria (Petersen et al., 1999). When the results of all examinations were normal, patients were considered to have subjective complaints. The nondemented subjects group consisted of 20 patients with subjective memory complaints, 2 patients with a psychiatric disorder, and 2 patients with temporal epilepsy. Diagnoses were made by consensus in a multidisciplinary team. The study was approved by the local ethical review board and all subjects gave written informed consent.

2.2. Follow-up

At follow-up, patients were asked to undergo a second lumbar puncture (minimum interval 6 months). Within the MCI group, 21 patients remained stable, and 34 progressed to AD (McKhann et al., 1984), 3 to fronto-temporal lobar degeneration (FTLD; Neary et al., 1998), 2 to vascular dementia (VaD; Román et al., 1993), 1 to dementia with Lewy bodies (DLB; McKeith et al., 2005), and 1 was diagnosed with normal pressure hydrocephalus. Within the 24 nondemented subjects, 6 patients with subjective complaints progressed to MCI, 2 to AD, and 1 to vascular dementia, while 15 remained stable. We used the last available MMSE to estimate cognitive decline over time (MMSE at follow-up available in 19 nondemented, 55 MCI, and 56 AD subjects).

2.3. CSF analyses

CSF was obtained by lumbar puncture, using a 25-gauge needle, and collected in 10-mL polypropylene tubes. Within 2 hours, CSF samples were centrifuged at 1800*g* for 10 minutes at 4° C. CSF was aliquoted in polypropylene tubes of 0.5 or 1 mL and stored at −80° C until further analysis. To circumvent interassay variability, baseline and follow-up samples were analyzed in the same assay (Bouwman et al., 2006; Verwey et al., 2008). CSF Aβ42, tau, and ptau-181 were measured with Innotest Luminex (Bouwman et al., 2009). Intra-assay coefficients of variation (CV) were 5.1% for Aβ42, 3.4% for tau, and 4.1% for ptau-181. Inter-assay CV's were 5.6% at 55 pg/mL and 5.5% at 133 pg/mL for Aβ42, 5.9% at 75 pg/mL and 6.4% at 215 pg/mL for tau, and 4.4% at 30 pg/mL and 3.6% at 47 pg/mL for ptau-181 ($n = 10$). Aβ40 was measured with an in-house method (Verwey et al., 2009). The detection limit was 0.39 ng/mL (3 SD [standard deviations] above background; %CV $<$ 20%). For Aβ40 intra-assay CV was 1.9%, and interassay CV was 10.7% at 4.71 ng/mL and 4.7% at 9.56 ng/mL ($n = 10$). NfL was determined by enzyme-linked immunosorbent assay (ELISA) essentially as described before (Norgren et al., 2004), however the first antibody was replaced by the in-house produced anti-neurofilament monoclonal antibody, clone 4F8. The detection limit was 0.095 ng/mL. For NfL intra-assay CV was 9.5% and interassay CV was 27.5% at 4.78 ng/mL ($n = 9$). NfH was measured in an in-house developed multiplex assay. Activated beads from Qiagen (Hilden, Germany) were covalently immobilized with an anti-neurofilament monoclonal antibody (9C9 generously provided by Carsten Korth, Germany). After blocking Du-

rapore filter plates (HTS screening plates; Millipore, Billerica, MA, USA) with phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA)/0.05% Tween-20, 50 μL of standard (NFH; Progen, Heidelberg, Germany), controls, CSF samples, and blanks were incubated with a suspension of microspheres (2500 beads per well) coupled with the capturing antibody for 14–18 hours at 4° C on an orbital plate shaker (600 rpm). All further incubations were performed under continuous shaking (600 rpm) at room temperature. CSF samples were diluted 5 times in PBS/1% BSA/6 mM ethylenediaminetetraacetic acid (EDTA). After washing with PBS/1%BSA/0.05% Tween-20, the wells were incubated with 25 μL of 1:1000 diluted anti-neurofilament polyclonal antibody (#N4142, Sigma, The Netherlands). After washing, wells were incubated with 50 μL of 1:200 diluted Phycoerythrin-labeled donkey-anti-rabbit polyclonal antibody (Jackson ImmunoResearch, West Grove, PA, USA). The plate was washed again and 100 μL reading solution (Bio-plex Sheath fluid; Bio-Rad, Hercules, CA, USA) was applied. The resulting fluorescence intensity signal on the specific bead was read with Bio-Plex 200 System (Bio-Rad; 50–100 microspheres). All analyses were performed in duplicate and normalized. Detection limit for NfH was 8.84 pg/mL $\pm$ 0.61. Intra-assay CV was 5.7%, and interassay CV was 18.3.% at 1774 pg/mL and 23.9% at 31131 pg/mL (n = 9). The concentration of isoprostane (iPF2α-VI) was determined by liquid chromatography tandem mass spectrometry (LC-MS/MS). In brief, 0.1 mL of 2 ng/mL deuterated internal standard (8iPF$_{2\alpha}$-d4; Cayman Chemical, Ann Arbor, MI, USA; cat. 316300) was added to 0.5 mL CSF. Butylated hydroxytoluene (BHT) was added to a final concentration of 0.05% to prevent arachidonic acid from auto-oxidation during sample clean-up. Then 0.5 mL of 2.6 M potassium hydroxide (KOH) was added, and samples were incubated for 60 minutes at 40° C. Afterwards, formic acid (20%) was added to adjust the pH to approximately 4.5, and the sample was loaded onto a solid phase extraction column (Oasis HLB, Etten-Leur, The Netherlands; Roest et al., 2008). The eluate was taken to dryness under a stream of nitrogen at room temperature, and the sample was redissolved in 100 μL 10% acetonitrile in water. Isoprostane concentrations were quantified by a 4000 Qtrap (AB SCIEX, Toronto, Canada) mass spectrometer. To calculate the isoprostane concentration, the analyte/internal standard peak area ratio was compared with a standard curve from 2 to 16 ng/mL isoprostane (Cayman Chemical; cat. 16300). Intrarun CV was 4.8%, and interrun CV 7.6% at 199 ng/mL and 10.1% at 43 pg/mL (n = 17). Isoprostane was measured in 146 patients.

2.4. Statistical analysis

Differences between diagnosis categories were assessed using analysis of variance (ANOVA) with post hoc Bonferroni corrections or Fisher exact test when applicable. Pearson correlations were used to assess correlations between continuous variables. Age- and sex-adjusted linear mixed models were applied to assess baseline effects for diagnosis and changes over time in CSF biomarkers by diagnosis. The CSF biomarkers (Aβ40, Aβ42, tau, ptau-181, NfL, NfH, and isoprostane) were the dependent variables (each in separate model), while diagnosis (treated as categorical variable) and time (in years; treated as a continuous variable) and interaction between diagnosis and time were independent variables. Diagnosis categories were recoded to be able to estimate β standard errors (βSE) for all diagnosis categories. A random intercept and random slope with time were assumed. For visualization purposes and to allow comparison of the effect sizes of the different CSF biomarkers, standardized β's were calculated with the formula β biomarker $\times$ SD time/SD biomarker. Additional analyses were performed to analyze change in CSF biomarker levels over time in relation to progression of MCI. For this analysis patients with stable MCI and patients that progressed from MCI to AD were included. An age- and sex-adjusted mixed model was used with CSF biomarkers (each in separate model) as dependent variable and MCI subgroup (dummies for MCI stable and MCI progression), time, and the interaction between MCI subgroups and time as independent variables. Finally, for biomarkers that showed change over time, we calculated the annualized changes of CSF biomarker level and MMSE, with the formula value of measurement at follow-up minus value of measurement at baseline, divided by follow-up period in years. Associations between annualized change in CSF biomarker levels and annualized change in MMSE score were assessed using linear regression models (data available for 130 patients), adjusted for sex, age, and diagnosis. Statistical significance was set at $p \leq 0.05$.

Table 1
Patient characteristics for the separate diagnosis categories

	Nondemented (n = 24)	MCI (n = 62)	AD (n = 68)
Age (years)	64 ± 10	68 ± 8	65 ± 7
Sex, n (%) female	7 (29%)	23 (37%)	31 (46%)
Follow-up time (years)	2.5 ± 1.7	2.0 ± 1.1	1.9 ± 1.0
MMSE baseline	28 ± 2	26 ± 2	22 ± 5*,**
MMSE follow-up[a]	27 ± 3	22 ± 5*	16 ± 7*,**
MMSE follow-up time (years)	4.0 ± 2.7	3.8 ± 2.1	3.2 ± 2.0
Medical history			
TIA or stroke, n (%)	3 (13%)	7 (11%)	2 (3%)
Hypertension, n (%)	8 (33%)	16 (26%)	12 (18%)
Hypercholesterolemia, n (%)	5 (21%)	7 (11%)	8 (12%)
Diabetes Mellitus, n (%)	1 (4%)	5 (8%)	1 (2%)

Data are represented as mean ± SD unless indicated otherwise.
Key: AD, Alzheimer's disease; MCI, mild cognitive impairment; MMSE, Mini Mental State Examination; SD, standard deviation; TIA, transient ischemic attack.
[a] Follow-up MMSE was available for 130 patients, and follow-up period was generally longer than for lumbar puncture.
* $p < 0.005$ versus nondemented subjects.
** $p < 0.005$ versus MCI.

3. Results

Table 1 presents the patient characteristics according to diagnosis. Patients with AD had a lower score on baseline MMSE, compared with nondemented subjects and MCI patients. Mean (SD) annual change in MMSE was −0.4 (1.0) for nondemented patients, −1.2 (1.9) for MCI patients and −2.2 (1.9) for AD patients. At baseline there were no correlations between CSF biomarker levels and storage time, but levels of isoprostane ($r = 0.20$; $p < 0.05$) and NfH ($r = 0.39$; $p < 0.001$) were positively correlated with age.

We used linear mixed models to investigate the effects of diagnosis and time on CSF biomarker levels of Aβ42, tau, ptau-181, isoprostane, NfH, NfL, and Aβ40. Age- and sex-adjusted analyses were performed with diagnosis as categorical variable and time as continuous variable, with an interaction term for diagnosis × time. There was a main effect of diagnosis for Aβ42, tau, ptau-181, and Aβ40 at baseline, whereas the biomarkers isoprostane, NfH, and NfL did not differentiate between diagnosis groups at baseline (Table 2).

Change in CSF biomarker levels over time, was found for isoprostane, NfH, NfL, Aβ40, and tau, but not for Aβ42 or ptau-181. Estimated annual changes by diagnosis group are represented in Table 2 (no significant interactions diagnosis × time). Levels of CSF isoprostane increased over time in all diagnosis groups, with an estimated annual increase in isoprostane levels of 1.9 pg/mL in nondemented patients, 2.3 pg/mL in MCI patients, and 1.9 pg/mL in AD patients. Levels of NfL decreased over time in AD and MCI patients, but not in nondemented subjects. Levels of NfH decreased over time, albeit nonsignificantly in AD patients, but not in MCI patients or nondemented subjects. CSF levels of Aβ40 increased in all patient groups, and CSF levels of tau increased in MCI and AD patients. Fig. 1 shows the standardized β's of the CSF biomarkers that showed change over time, to allow comparison of effect sizes; isoprostane had the largest effect size.

Secondly, we examined whether disease progression in MCI patients was associated with change in biomarker levels. We analyzed the effect of MCI subgroups (stable vs. progressing to AD) on the change of CSF biomarker levels over time. There were baseline effects of MCI subgroups for CSF Aβ42, tau, and ptau-181 (all $p < 0.005$), but not for the other, nonspecific CSF biomarkers. By contrast, for isoprostane and for Aβ40 we found that change of levels over time

Table 2
Baseline levels and change over time of CSF biomarker levels

	Nondemented ($n = 24$)	MCI ($n = 62$)	AD ($n = 68$)
Aβ42 (pg/mL), baseline	403 ± 125	307 ± 114**	263 ± 83**,***
Aβ42 (pg/mL), follow-up	399 ± 135	315 ± 119	273 ± 78
Annual change, β (SE)	−1.9 (4.3)	−0.4 (3.5)	5.0 (3.7)
Tau (pg/mL), baseline	104 ± 59	155 ± 109*	156 ± 87**
Tau (pg/mL), follow-up	121 ± 87	172 ± 122	189 ± 89
Annual change, β (SE)	4.9 (3.4)	5.8 (2.6)	5.6 (2.7)
Ptau-181 (pg/mL), baseline	31 ± 17	42 ± 29	43 ± 26*
Ptau-181 (pg/mL), follow-up	32 ± 13	45 ± 31	48 ± 24
Annual change, β (SE)	−0.2 (1.2)	0.7 (1.0)	0.0 (1.1)
Isoprostane (pg/mL), baseline[a]	15.2 ± 4.7	15.7 ± 5.0	14.6 ± 4.3
Isoprostane (pg/mL), follow-up	19.8 ± 9.8	20.3 ± 8.6	18.1 ± 7.7
Annual change, β (SE)	1.9 (0.9)	2.3 (0.5)	1.9 (0.5)
NfL (ng/mL), baseline	5.0 ± 4.6	5.4 ± 4.6	5.6 ± 4.4
NfL (ng/mL), follow-up	5.4 ± 5.9	4.0 ± 4.0	3.9 ± 3.5
Annual change, β (SE)	−0.18 (0.50)	−0.79 (0.31)	−0.96 (0.31)
NfH (pg/mL), baseline	404 ± 147	471 ± 206	475 ± 316
NfH (pg/mL), follow-up	412 ± 174	458 ± 185	422 ± 178
Annual change, β (SE)	−1.5 (19.9)	−5 (13)	−24 (13)
Aβ40 (ng/mL), baseline	9.6 ± 3.0	9.5 ± 3.2	8.5 ± 2.8*
Aβ40 (ng/mL), follow-up	11.0 ± 2.9	10.0 ± 3.1	9.2 ± 3.4
Annual change, β (SE)	0.61 (0.22)	0.28 (0.14)	0.43 (0.14)

Data are represented as mean ± SD or β (SE). Linear mixed models were applied to assess the associations between diagnosis, baseline CSF biomarkers and change in CSF biomarker levels over time (in years). A random intercept and a random slope with time were assumed. Age and sex adjusted analyses were performed with diagnosis as categorical variable and time as continuous variable, with an interaction term for diagnosis × time. β and p values were calculated with the linear mixed model. In this model, the main effect of diagnosis represents the group differences at baseline, and the main effect of time represents the annual change of biomarker levels for each diagnostic category. The interaction of diagnosis × time was not significant for any of the markers, implying that the estimated time effects did not differ significantly by diagnosis.

Key: Aβ40, amyloid-beta n-40; Aβ42, amyloid-beta 1-42; AD, Alzheimer's disease; CSF, cerebrospinal fluid; iPF2α-VI, isoprostane; MCI, mild cognitive impairment; NfH, neurofilaments heavy; NfL, neurofilaments light; ptau-181, tau phosphorylated at threonine 181; SE, standard error; tau, total tau.

[a] For isoprostane data was available for 21 nondemented patients, 61 MCI patients and 64 AD patients.

* $p < 0.05$ vs. nondemented subjects.

** $p < 0.005$ vs. nondemented subjects.

*** $p < 0.005$ vs. MCI patients.

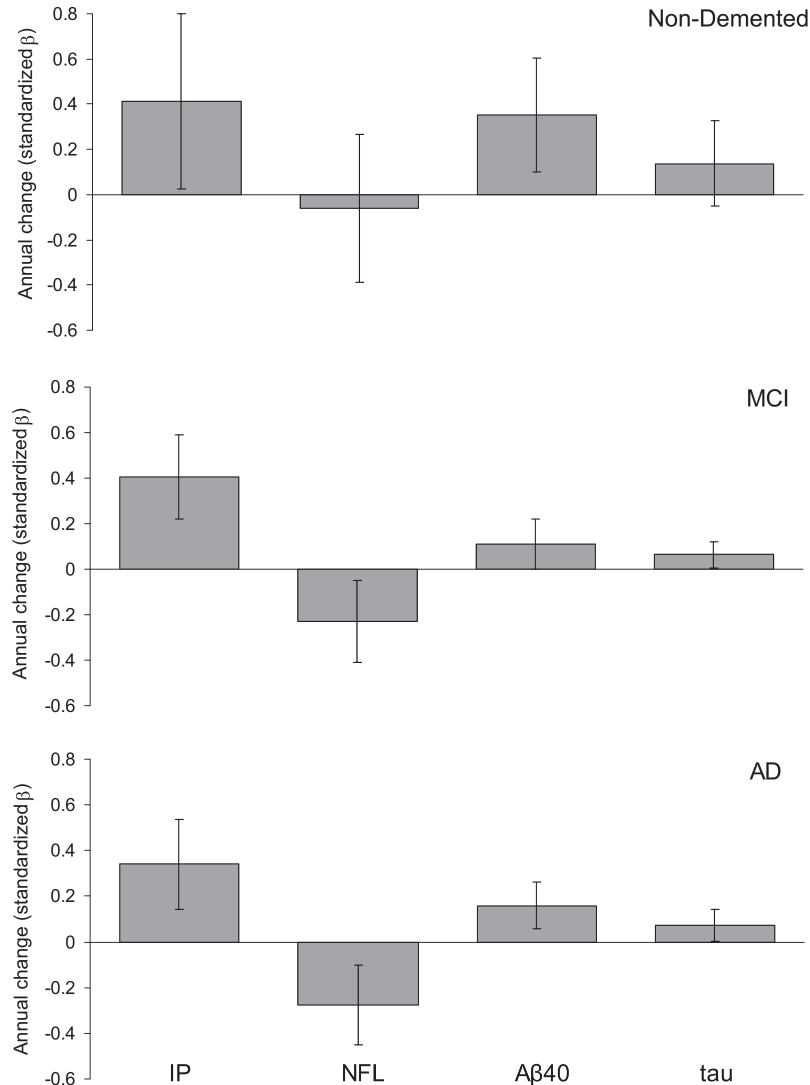

Fig. 1. Standardized time effects of the CSF biomarkers by diagnosis. Bars represent change in biomarker level over time (standardized β with 95% confidence interval [CI]). Linear mixed models were applied to assess the associations between diagnosis, baseline CSF biomarkers, and change in CSF biomarker levels (β) over time (in years). A random intercept and a random slope with time were assumed. Age- and sex-adjusted analyses were performed with diagnosis as categorical variable and time as continuous variable, with an interaction term for diagnosis $\times$ time. Standardized β values were calculated with the formula β biomarker $\times$ SD time/SD biomarker, to estimate and compare the effect sizes of the different CSF biomarkers. Aβ40, amyloid-beta n-40; AD, Alzheimer's disease; CSF, cerebrospinal fluid; IP, ; isoprostane, iPF2α-VI; MCI, mild cognitive impairment; NFL, neurofilaments light; tau, total tau.

were different between MCI stable and MCI progressive patients (p for interaction both < 0.05), whereas for the other CSF biomarkers effects were the same for MCI stable and progressive patients. Isoprostane levels increased in MCI progressive patients (β [SE] 3.8 [0.8]), while CSF isoprostane levels hardly changed in stable MCI patients (β [SE] 0.9 [1.0]), as shown in Fig. 2. Contrary to our expectations, Aβ40 levels increased in MCI stable patients (β [SE] 0.55 [0.16]), but not in MCI progressors (β [SE] 0.07 [0.12]).

Next, we investigated whether change in CSF biomarker levels over time was associated with cognitive decline, as an indicator of clinical disease progression. For all biomarkers that showed significant change over time, we performed linear regression analyses to evaluate the association of change in CSF biomarker levels with change of MMSE over time. Annual change in isoprostane levels was associated with annual MMSE change, as shown in Fig. 3: β (SE) were -0.11 (0.06) in nondemented (n=16; $p = 0.08$), -0.11 (0.05) in MCI ($n = 54$; $p < 0.05$) and -0.11 (0.04) in AD ($n = 53$; $p < 0.05$). For the other CSF biomarkers, there were no associations with cognitive decline: β (SE) of NfL were -0.11 (0.17) for nondemented ($n = 19$), -0.10 (0.09) for MCI ($n = 55$) and -0.11 (0.10) for AD ($n = 56$); for Aβ40 -0.23 (0.32), 0.06 (0.29), and -0.03 (0.21); for tau -0.010 (0.13), 0.008 (0.009), and 0.004 (0.010); all $p >$ 0.05.

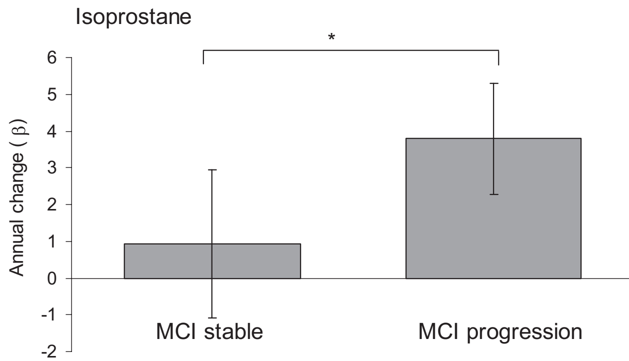

Fig. 2. Time effects by mild cognitive impairment (MCI) subgroups. Bars represent change in isoprostane over time (β with 95% confidence interval [CI]). A linear mixed model was used with cerebrospinal fluid (CSF) isoprostane as dependent variable and age, sex, time, MCI subgroup (dummies for MCI stable and MCI progression), and interaction terms (dummy MCI subgroup $\times$ time) were entered as covariates. There was a significant interaction of MCI subgroup $\times$ time.

4. Discussion

We found that 4 nonspecific CSF biomarkers, i.e., isoprostane, Aβ40, tau, and NfL, showed change over time in AD. Of these biomarkers, isoprostane appeared to be the most promising marker for monitoring of disease progression, because it had the largest effect size. In addition, increase over time of isoprostane levels was associated with progression of MCI to AD, and increase of isoprostane was associated with cognitive decline over time.

With the development of symptom-modifying drugs it is of utmost importance to find biomarkers that allow for the monitoring of disease progression of AD. Most previous longitudinal studies examined only the CSF biomarkers Aβ42, tau, and ptau-181 (Blennow et al., 2007; Bouwman et al., 2007; Buchhave et al., 2009; Li et al., 2007; Mollenhauer et al., 2005). Longitudinal effects of these specific markers were disappointing, as was also illustrated by a recent meta-analysis of these studies (Zhou et al., 2009). In the current study we used Luminex technology for the longitudinal measurement which did not seem to be an added value over enzyme-linked immunosorbent assay, as there were no changes in CSF Aβ42 and ptau-181 levels over time, while CSF tau levels increased only minimally with disease progression. These findings are in line with the idea that these AD-specific markers are state markers (Perrin et al., 2009).

In the current study, we found that isoprostane, NfH, NfL, Aβ40, and tau were all associated with disease progression in AD, with the strongest effect for isoprostane. Furthermore, increase of isoprostane levels was associated with progression of MCI to AD and cognitive decline, as measured by repeated MMSE. The findings of our study are supported by a few small studies in MCI and AD patients that also showed increase of isoprostane over time (Brys et al., 2009; de Leon et al., 2007; Quinn et al., 2004). Isoprostane is a marker of membrane lipid peroxidation and inflammation and previous data suggested that an increase of CSF isoprostane levels in cognitively declining patients reflected progressive neuronal oxidative stress and progression of neurodegenerative changes (Pratico and Sung, 2004).

NfL as measured with the current assay (and to a lesser extent also NfH) decreased over time in MCI and AD, but not in nondemented patients. However, we were not able to establish a relation with cognitive decline as measured with MMSE. Possibly, the decrease in neurofilament levels over time reflects the presence and progression of atrophy. Our findings should be examined in relation to MRI results and postmortem findings in future studies.

Aβ40 increased over time in our study. Former studies that examined longitudinal effects of Aβ40 found no effect over time, but these studies included small patient groups and used a different test for the measurement of Aβ40 (Brys et al., 2009; Kanai et al., 1999; Mollenhauer et al., 2005). The increase in Aβ40 levels possibly reflects the increase of solid, less diffuse, types of amyloid plaques, that generally develop in later stages of AD (Iwatsubo et al., 1994). Unexpectedly, in MCI patients we found the largest increase in Aβ40 levels in those patients that did not progress to AD. Possibly, in these stable MCI patients there was an in-

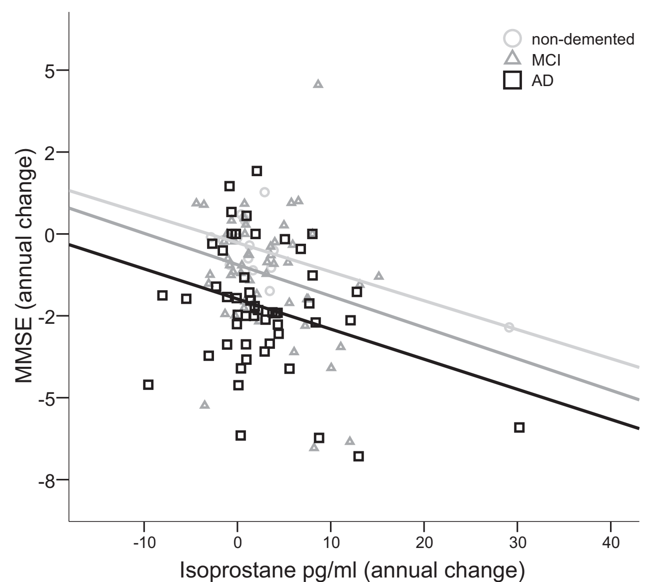

Fig. 3. Associations of change in isoprostane levels over time and cognitive decline. Associations between annualized change in cerebrospinal fluid (CSF) isoprostane levels and annualized change in Mini Mental State Examination (MMSE) score were assessed using linear regression models (data available for 130 patients), adjusted for sex, age, and diagnosis. We calculated the annualized change of CSF biomarker level and MMSE, with the formula: value of measurement at follow-up minus value of measurement at baseline, divided by follow-up period in years. The light gray line and circles represent nondemented subjects (β [SE] -0.11 [0.06]), the dark gray line and triangles represent mild cognitive impairment (MCI) patients (β [SE] -0.11 [0.05]), and the black line and squares represent Alzheimer's disease (AD) patients (β [SE] -0.11 [0.04]).

creased synthesis of beta-amyloid (Aβ), in combination with a lack of formation of compact Aβ plaques, as seen in AD. However, the rise in Aβ40 levels may also be unrelated to AD pathology, and related to for instance vascular pathology, which is not necessarily related to clinical deterioration. The current study was not designed for analyzing relations between biomarker level change, disease progression, and vascular pathology. Further studies could examine correlations between changes in biomarker levels and increase of for instance white matter hyperintensities (WMH) at follow-up MRI.

Lastly, CSF tau levels showed some increase over time during disease progression. CSF tau has been suggested to reflect the degree of neuronal cell death and to be a more general marker for neuronal damage (Arai et al., 1997; Buerger et al., 2006; Hesse et al., 2001; Riemenschneider et al., 2003; van der Vlies et al., 2009). Effects, however, were modest and the cross-sectional difference between diagnosis groups exceeded by far the longitudinal changes within individuals.

We studied a panel of potential CSF biomarkers to monitor disease progression in a large group of memory clinic patients. Most of these biomarkers have only been evaluated in small patient groups before, and some have never been studied serially in AD. Our study allowed comparison of all biomarkers, because they were measured in the same patients at the same time points. We have included patients of all stages of the AD disease spectrum, including nondemented subjects, MCI patients, and AD patients. We had expected that effects would have been diagnosis-specific for more biomarkers. The fact that there were no diagnosis-specific effects seemingly cannot be explained by age. First, there were only correlations between age and biomarker level for isoprostane and NfL. Second, all analyses were adjusted for age. A potential explanation could be that the group of nondemented subjects ($n = 24$) was too small in comparison to the groups of MCI ($n = 62$) and AD patients ($n = 68$) for adequate analyses, which could be considered a limitation. An alternative explanation could be that the group of nondemented subjects were in a prodromal stage of AD. Some of the patients in the group of nondemented subjects developed MCI or even dementia during follow-up. The latter explanation is supported by our finding that isoprostane increase was associated with a decrease in MMSE score, even in the nondemented subjects. For further studies we aim to include a larger group of controls, preferably community dwelling volunteers with both cognitive and biomarker follow-up.

These results imply that once a patient has developed the core AD pathological hallmarks of amyloid plaques and tangles to a certain extent, other nonspecific processes like neuronal cell and synaptic loss, as well as oxidative stress are characteristic for disease progression. These less specific processes are the disease processes to be monitored in studies focusing on disease progression. The results of this kind of studies could possibly also provide suggestions for treatment options for later stages of AD pathology. It could be hypothesized that therapies that focus on reducing the nonspecific pathogenic process, instead of intervening in amyloid accumulation, could be of benefit for patients at the stage of clinical AD.

Disclosure statement

Prof. Scheltens has served as consultant for Wyeth-Elan, Genentech, Danone, and Novartis, and received funding for travel from Pfizer, Elan, Janssen, and Danone Research. Dr. Teunissen has served as board member for Innogenetcs and the NeuroAdvisory Board for which she received consultancy fees. Drs. Kester, Scheffer, Koel-Simmelink, Twaalfhoven, Verwey, Veerhuis, Bouwman, Van der Flier, and Profs. Twisk and Blankenstein report no disclosures.

The study was approved by the local ethical review board and all subjects gave written informed consent.

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References

Arai, H., Morikawa, Y., Higuchi, M., Matsui, T., Clark, C.M., Miura, M., Machida, N., Lee, V.M., Trojanowski, J.Q., Sasaki, H., 1997. Cerebrospinal fluid tau levels in neurodegenerative diseases with distinct tau-related pathology. Biochem. Biophys. Res. Commun. 236, 262–264.

Blennow, K., Hampel, H., 2003. CSF markers for incipient Alzheimer's disease. Lancet Neurol. 2, 605–613.

Blennow, K., Zetterberg, H., Minthon, L., Lannfelt, L., Strid, S., Annas, P., Basun, H., Andreasen, N., 2007. Longitudinal stability of CSF biomarkers in Alzheimer's disease. Neurosci. Lett. 419, 18–22.

Bouwman, F.H., Schoonenboom, N.S., Verwey, N.A., van Elk, E.J., Kok, A., Blankenstein, M.A., Scheltens, P., van der Flier, W.M., 2009. CSF biomarker levels in early and late onset Alzheimer's disease. Neurobiol. Aging 30, 1895–1901.

Bouwman, F.H., van der Flier, W.M., Schoonenboom, N.S., van Elk, E.J., Kok, A., Rijmen, F., Blankenstein, M.A., Scheltens, P., 2007. Longitudinal changes of CSF biomarkers in memory clinic patients. Neurology 69, 1006–1011.

Bouwman, F.H., van der Flier, W.M., Schoonenboom, N.S., van Elk, E.J., Kok, A., Scheltens, P., Blankenstein, M.A., 2006. Usefulness of longitudinal measurements of beta-amyloid1-42 in cerebrospinal fluid of patients with various cognitive and neurologic disorders. Clin. Chem. 52, 1604–1606.

Brys, M., Pirraglia, E., Rich, K., Rolstad, S., Mosconi, L., Switalski, R., Glodzik-Sobanska, L., de, S.S., Zinkowski, R., Mehta, P., Pratico, D., Saint Louis, L.A., Wallin, A., Blennow, K., de Leon, M.J., 2009. Prediction and longitudinal study of CSF biomarkers in mild cognitive impairment. Neurobiol. Aging 30, 682–690.

Buchhave, P., Blennow, K., Zetterberg, H., Stomrud, E., Londos, E., Andreasen, N., Minthon, L., Hansson, O., 2009. Longitudinal study of CSF biomarkers in patients with Alzheimer's disease. PLoS. One. 4, e6294.

Buerger, K., Otto, M., Teipel, S.J., Zinkowski, R., Blennow, K., DeBernardis, J., Kerkman, D., Schroder, J., Schonknecht, P., Cepek, L., McCulloch, C., Moller, H.J., Wiltfang, J., Kretzschmar, H., Hampel, H., 2006. Dissociation between CSF total tau and tau protein phosphorylated at threonine 231 in Creutzfeldt-Jakob disease. Neurobiol. Aging 27, 10–15.

de Jong, D., Kremer, B.P., Olde Rikkert, M.G., Verbeek, M.M., 2007. Current state and future directions of neurochemical biomarkers for Alzheimer's disease. Clin. Chem. Lab Med. 45, 1421–1434.

de Leon, M.J., Mosconi, L., Li, J., de, S.S., Yao, Y., Tsui, W.H., Pirraglia, E., Rich, K., Javier, E., Brys, M., Glodzik, L., Switalski, R., Saint Louis, L.A., Pratico, D., 2007. Longitudinal CSF isoprostane and MRI atrophy in the progression to AD. J. Neurol. 254, 1666–1675.

Hesse, C., Rosengren, L., Andreasen, N., Davidsson, P., Vanderstichele, H., Vanmechelen, E., Blennow, K., 2001. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci. Lett. 297, 187–190.

Iwatsubo, T., Odaka, A., Suzuki, N., Mizusawa, H., Nukina, N., Ihara, Y., 1994. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). Neuron 13, 45–53.

Jack, C.R., Jr., Knopman, D.S., Jagust, W.J., Shaw, L.M., Aisen, P.S., Weiner, M.W., Petersen, R.C., Trojanowski, J.Q., 2010. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 9, 119–128.

Kanai, M., Shizuka, M., Urakami, K., Matsubara, E., Harigaya, Y., Okamoto, K., Shoji, M., 1999. Apolipoprotein E4 accelerates dementia and increases cerebrospinal fluid tau levels in Alzheimer's disease. Neurosci Lett. 267, 65–68.

Kumar-Singh, S., 2008. Cerebral amyloid angiopathy: pathogenetic mechanisms and link to dense amyloid plaques. Genes Brain Behav. 7 Suppl 1, 67–82.

Li, G., Sokal, I., Quinn, J.F., Leverenz, J.B., Brodey, M., Schellenberg, G.D., Kaye, J.A., Raskind, M.A., Zhang, J., Peskind, E.R., Montine, T.J., 2007. CSF tau/Abeta42 ratio for increased risk of mild cognitive impairment: a follow-up study. Neurology 69, 631–639.

McKeith, I.G., Dickson, D.W., Lowe, J., Emre, M., O'Brien, J.T., Feldman, H., Cummings, J., Duda, J.E., Lippa, C., Perry, E.K., Aarsland, D., Arai, H., Ballard, C.G., Boeve, B., Burn, D.J., Costa, D., Del, S.T., Dubois, B., Galasko, D., Gauthier, S., Goetz, C.G., Gomez-Tortosa, E., Halliday, G., Hansen, L.A., Hardy, J., Iwatsubo, T., Kalaria, R.N., Kaufer, D., Kenny, R.A., Korczyn, A., Kosaka, K., Lee, V.M., Lees, A., Litvan, I., Londos, E., Lopez, O.L., Minoshima, S., Mizuno, Y., Molina, J.A., Mukaetova-Ladinska, E.B., Pasquier, F., Perry, R.H., Schulz, J.B., Trojanowski, J.Q., Yamada, M., 2005. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 65, 1863–1872.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., Stadlan, E.M., 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34, 939–944.

Mollenhauer, B., Bibl, M., Trenkwalder, C., Stiens, G., Cepek, L., Steinacker, P., Ciesielczyk, B., Neubert, K., Wiltfang, J., Kretzschmar, H.A., Poser, S., Otto, M., 2005. Follow-up investigations in cerebrospinal fluid of patients with dementia with Lewy bodies and Alzheimer's disease. J Neural Transm. 112, 933–948.

Montine, T.J., Quinn, J.F., Montine, K.S., Kaye, J.A., Breitner, J.C., 2005. Quantitative in vivo biomarkers of oxidative damage and their application to the diagnosis and management of Alzheimer's disease. J Alzheimers Dis. 8, 359–367.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S., Freedman, M., Kertesz, A., Robert, P.H., Albert, M., Boone, K., Miller, B.L., Cummings, J., Benson, D.F., 1998. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51, 1546–1554.

Norgren, N., Rosengren, L., Stigbrand, T., 2003. Elevated neurofilament levels in neurological diseases. Brain Res. 987, 25–31.

Norgren, N., Sundstrom, P., Svenningsson, A., Rosengren, L., Stigbrand, T., Gunnarsson, M., 2004. Neurofilament and glial fibrillary acidic protein in multiple sclerosis. Neurology 63, 1586–1590.

Perrin, R.J., Fagan, A.M., Holtzman, D.M., 2009. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature 461, 916–922.

Petersen, R.C., Smith, G.E., Waring, S.C., Ivnik, R.J., Tangalos, E.G., Kokmen, E., 1999. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol. 56, 303–308.

Petzold, A., 2005. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci. 233, 183–198.

Pijnenburg, Y.A., Janssen, J.C., Schoonenboom, N.S., Petzold, A., Mulder, C., Stigbrand, T., Norgren, N., Heijst, H., Hack, C.E., Scheltens, P., Teunissen, C.E., 2007. CSF neurofilaments in frontotemporal dementia compared with early onset Alzheimer's disease and controls. Dement Geriatr Cogn Disord. 23, 225–230.

Pratico, D., Sung, S., 2004. Lipid peroxidation and oxidative imbalance: early functional events in Alzheimer's disease. J Alzheimers Dis. 6, 171–175.

Quinn, J.F., Montine, K.S., Moore, M., Morrow, J.D., Kaye, J.A., Montine, T.J., 2004. Suppression of longitudinal increase in CSF F2-isoprostanes in Alzheimer's disease. J Alzheimers Dis. 6, 93–97.

Riemenschneider, M., Wagenpfeil, S., Vanderstichele, H., Otto, M., Wiltfang, J., Kretzschmar, H., Vanmechelen, E., Forstl, H., Kurz, A., 2003. Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. Mol Psychiatry 8, 343–347.

Roest, M., Voorbij, H.A.M., Van der Schouw, Y.T., Peeters, P.H.M., Teerlink, T., Scheffer, P.G., 2008. High levels of urinary F2-isoprostanes predict cardiovascular mortality in postmenopausal women. J Clin Lipidol. 2, 298–303.

Román, G.C., Tatemichi, T.K., Erkinjuntti, T., Cummings, J.L., Masdeu, J.C., Garcia, J.H., Amaducci, L., Orgogozo, J.M., Brun, A., Hofman, A., et al., 1993. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 43, 250–260.

van der Vlies, A.E., Verwey, N.A., Bouwman, F.H., Blankenstein, M.A., Klein, M., Scheltens, P., van der Flier, W.M., 2009. CSF biomarkers in relationship to cognitive profiles in Alzheimer disease. Neurology 72, 1056–1061.

Verwey, N.A., Bouwman, F.H., van der Flier, W.M., Veerhuis, R., Scheltens, P., Blankenstein, M.A., 2008. Variability in longitudinal cerebrospinal fluid tau and phosphorylated tau measurements. Clin Chem Lab Med. 46, 1300–1304.

Verwey, N.A., Veerhuis, R., Twaalfhoven, H.A., Wouters, D., Hoozemans, J.J., Bollen, Y.J., Killestein, J., Bibl, M., Wiltfang, J., Hack, C.E., Scheltens, P., Blankenstein, M.A., 2009. Quantification of amyloid-beta 40 in cerebrospinal fluid. J Immunol Methods 348, 57–66.

Zhou, B., Teramukai, S., Yoshimura, K., Fukushima, M., 2009. Validity of cerebrospinal fluid biomarkers as endpoints in early-phase clinical trials for Alzheimer's disease. J Alzheimers Dis. 18, 89–102.