SERIAL CSF SAMPLING IN ALZHEIMER’S DISEASE: SPECIFIC VERSUS NON-SPECIFIC MARKERS
CHAPTER 4.2

ABSTRACT

Objective: In this longitudinal study we aimed to investigate the change over time in 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), in an attempt to identify biomarkers that reflect the course of Alzheimer’s disease (AD).

Methods: Twenty-four non-demented subjects, 62 patients with mild cognitive impairment (MCI) and 68 AD patients underwent two lumbar punctures at our memory clinic, with minimum interval of 6 and a mean±SD of 24±13 months. CSF biomarker levels were measured in baseline and follow-up samples, and linear mixed models were used to assess change over time. In addition, CSF biomarker levels were related to clinical outcomes including conversion to AD and change in MMSE.

Results: AD-specific CSF biomarkers, Aβ42, tau and ptau-181, differentiated between diagnosis groups (p<0.05), whereas isoprostane, NfH and NFL did not. In contrast, effects of follow-up time were only found for non-specific CSF biomarkers: levels of NFL decreased, and levels of isoprostane, Aβ40 and tau increased over time (p<0.05). Isoprostane showed the largest increase over time. In addition, increase in isoprostane was associated with progression of MCI to AD, and with cognitive decline as reflected by change in MMSE.

Discussion: Contrary to AD-specific markers, non-specific CSF biomarkers, most notably isoprostane, showed change over time. These markers could potentially be used to monitor disease progression in AD.

Maartje I. Kester MD
Peter G. Scheffer PhD
Marleen J. Koel-Simmelink PhD, Harry Twaalfhoven
Nicolaas A. Verwey
Rob Veerhuis PhD
Jos W. Twisk PhD
Femke H. Bouwman MD PhD
Marinus A. Blankenstein PhD
Philip Scheltens MD PhD
Charlotte Teunissen PhD
Wiesje M. van der Flier PhD

1 Alzheimer Center and Department of Neurology, 2 Department of Clinical Chemistry, 3 Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands, 4 Department of Neurology, Catharina Hospital, Eindhoven, The Netherlands

Submitted
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. 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. 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.

The specific biomarkers, Aβ42, tau and ptau-181, seem less suitable as biomarkers for monitoring of disease progression. Amyloid plaque deposition and tau tangle formation are early processes in AD, that may show little or no change later on. Other, more general and thus less specific disease processes are increasingly considered to play a major role in advanced stages of the disease. 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. 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. 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. Few cross-sectional studies have shown increased levels of neurofilaments in AD 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, since Aβ40 has been associated with solid, less diffuse, types of amyloid plaques, that generally develop in later stages of AD.

We aimed to assess longitudinal effects of CSF biomarkers, in order to identify biomarkers that are useful to monitor disease progression. Our panel of seven 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 MMSE, in a large cohort of AD and mild cognitive impairment (MCI) patients and non-demented subjects.

METHODS

Patients

We included patients with AD (n=68), MCI (n=62) and non-demented subjects (n=24) with CSF at two time points. At baseline all patients underwent standard dementia screening including physical and neurological examination, laboratory tests, EEG and MRI. Cognitive screening included a Mini-Mental State Examination (MMSE), but usually involved comprehensive neuropsychological testing. The diagnosis of...
probable AD was made according to NINCDS-ADRDA criteria. The diagnosis of MCI was made according to Petersen’s criteria. When the results of all examinations were normal, patients were considered to have subjective complaints. The non-demented 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.

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, 3 to fronto-temporal lobar degeneration (FTLD), 2 to vascular dementia (VaD), 1 to dementia with Lewy Bodies (DLB) and 1 was diagnosed with normal pressure hydrocephalus. Within the 24 non-demented subjects, 6 patients with subjective complaints progressed to MCI, 2 to AD and 1 to VaD, while 15 remained stable. We used the last available MMSE to estimate cognitive decline over time (MMSE at follow-up available in 19 non-demented, 55 MCI and 56 AD).

CSF analyses
CSF was obtained by lumbar puncture, using a 25-gauge needle, and collected in 10 ml polypropylene tubes. Within two hours, CSF samples were centrifuged at 1800g for 10 minutes at 4°C. CSF was aliquoted in polypropylene tubes of 0.5 or 1ml and stored at -80°C until further analysis. To circumvent inter-assay variability, baseline and follow-up samples were analyzed in the same assay. CSF Aβ42, tau and ptau-181 were measured with Innoltest Luminex. 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. The detection limit was 0.39 ng/ml (3SD above background/%CV<20%). For Aβ40 intra-assay CV was 1.9%, and inter-assay CV was 10.7% at 4.71 ng/ml and 4.7% at 9.56 ng/ml (n=10). NFL was determined by ELISA essentially as described before, 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 inter-assay 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 Durapore filter plates (HTS screening plates, Millipore) with PBS/1%BSA/0.05% Tween-20 and 50 µl of standard (NFH, Progen), controls, CSF samples, and blanks were incubated with a suspension of microspheres (2500 beads/well) coupled with the capturing antibody for 14-18 hours at 4°C on an orbital plate shaker (600rpm). All further incubations were performed under continuous shaking (600rpm) at room temperature. CSF samples were diluted 5 times in PBS/1% BSA/6 mM 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 Phycocyetrin labelled donkey-anti-rabbit-polyclonal antibody (Jackson ImmunoResearch). The plate was washed again and 100 µl reading solution (Bio-plex Sheath fluid) 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 ± 0.61. Intra-assay CV was 5.7%, and inter-assay CV was 18.3% at 1774 pg/ml and 23.9% at 3113 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 (8iPF2α-d4; Cayman chemical, US; 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 KOH was added, and samples were incubated for 60 min at 40°C. Afterwards, formic acid (20%) was added to adjust the pH to ~4.5, and the sample was loaded onto a solid phase extraction column (Oasis HLB, Waters, US). 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 (PE Sciex, Canada) mass spectrometer. To calculate the isoprostane concentration, the analyte/internal standard peak area ratio was compared with a standard curve from 2-16 ng/ml isoprostane (Cayman; cat. 16300). Intra-run CV was 4.8%, and inter-run CV 7.6% at 199 ng/ml and 10.1% at 43 pg/ml (n=17). Isoprostane was measured in 146 patients.

Statistical analysis

Differences between diagnosis categories were assessed using ANOVA with post hoc Bonferroni corrections or Fisher exact test when applicable. Pearson’s 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(SE)’s 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*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
CHAPTER 4.2

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≤0.05.

RESULTS

Table 1 presents the patient characteristics according to diagnosis. Patients with AD had a lower score on baseline MMSE, compared to non-demented subjects and MCI patients. Mean (SD) annual change in MMSE was -0.4(1.0) for non-demented 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 non-demented 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 non-demented subjects. Levels of NfH decreased over time, albeit non-significantly, in AD patients, but not in MCI patients or non-demented subjects. CSF levels of Aβ40 increased in all patients groups, and CSF levels of tau increased in MCI and AD patients. Figure 1 shows the standardized β’s

Table 1. Patient characteristics for the separate diagnosis categories.

<table>
<thead>
<tr>
<th></th>
<th>Non-demented (n=24)</th>
<th>MCI (n=62)</th>
<th>AD (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 10</td>
<td>68 ± 8</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Sex, n (%) female</td>
<td>7 (29%)</td>
<td>23 (37%)</td>
<td>31 (46%)</td>
</tr>
<tr>
<td>Follow-up time (years)</td>
<td>2.5 ± 1.7</td>
<td>2.0 ± 1.1</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>MMSE baseline</td>
<td>28 ± 2</td>
<td>26 ± 2</td>
<td>22 ± 5 * *</td>
</tr>
<tr>
<td>MMSE follow-up ~</td>
<td>27 ± 3</td>
<td>22 ± 5 *</td>
<td>16 ± 7 * *</td>
</tr>
<tr>
<td>MMSE follow-up time (years)</td>
<td>4.0 ± 2.7</td>
<td>3.8 ± 2.1</td>
<td>3.2 ± 2.0</td>
</tr>
</tbody>
</table>

Data are represented as mean±SD unless indicated otherwise. ~ Follow-up MMSE was available for 130 patients, and follow-up period was generally longer than for lumbar puncture. * p<0.005 versus non-demented subjects, * * p<0.005 versus MCI.
## Table 2. Baseline levels and change over time of CSF biomarker levels

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

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’s 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.

* p<0.05 vs non-demented subjects, ** p<0.005 vs non-demented subjects, † p<0.005 vs MCI patients. For isoprostane data was available for 21 non-demented patients, 61 MCI patients and 64 AD patients.
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, non-specific CSF biomarkers. By contrast, for isoprostane and for Aβ40 we found that change of levels over time 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 Figure 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 indicator of clinical disease progression. For all biomarkers that showed significant change over time, we performed linear regression

![Figure 1. Standardized time effects of the CSF biomarkers by diagnosis. Bars represent change in biomarker level over time (standardized β with 95% CI). Linear mixed models were applied to assess the associations between diagnosis, baseline CSF biomarkers and change in CSF biomarker levels over time (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*time. Standardized β’s were calculated with the formula β biomarker*SD time/SD biomarker, to estimate and compare the effect sizes of the different CSF biomarkers.](image-url)
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 Figure 3: $\beta$(SE) were -0.11(0.06) in non-demented (n=16, p=0.08), $\beta$(SE) 0.11 (0.05) in MCI (n=54, p<0.05) and $\beta$(SE) -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 non-demented (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.

DISCUSSION

We found that four non-specific 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, since 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. Longitudinal effects of these specific markers were disappointing, as was also illustrated by a recent meta-analysis of these studies. In the current study we used Luminex technology for the longitudinal measurement which did not seem to be an added value over ELISA, 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.

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. 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.

NFL (and to a lesser extent also NFH) decreased over time in MCI and AD, but not in non-demented 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 post-mortem 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. 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. 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 increased synthesis of 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 to vascular pathology, which is not necessarily related to clinical deterioration. 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. 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, since 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 non-demented subjects, MCI patients and AD patients. We had expected that effects would have been diagnosis specific for more biomarkers. One explanation could be that the group of non-demented 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 non-demented subjects included subjects that were in a prodromal stage of AD. Some of the patients in the group of non-demented 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 in the non-demented subjects.

These results imply that once a patient has developed the core AD pathological hallmarks of amyloid plaques and tangles to a certain extent, other non-specific 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 focussing 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 non-specific pathogenic process, instead of intervening in amyloid accumulation, could be of benefit for patients at the stage of clinical AD.
REFERENCES


