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

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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 ± SD of 24 ± 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.

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-

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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-