ELSEVIER

Alzheimer's & Dementia ■ (2015) 1-9

Alzheimer's & Dementia

C-terminal neurogranin is increased in cerebrospinal fluid but unchanged in plasma in Alzheimer's disease

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Abstract

Background: Biomarkers monitoring synaptic degeneration/loss would be valuable for Alzheimer's disease (AD) diagnosis. Postsynaptic protein neurogranin may be a promising cerebrospinal fluid (CSF) biomarker but has not yet been evaluated as a plasma biomarker.

Methods: Using an in-house designed prototype enzyme-linked immunosorbent assay (ELISA) targeting neurogranin C-terminally, we studied neurogranin in paired CSF/plasma samples of controls (n = 29) versus patients experiencing MCI, or dementia, due to AD (in total n = 59).

Results: CSF neurogranin was increased in AD and positively correlated with CSF tau, whereas there was a negative relationship between CSF neurogranin (and tau) and CSF $A\beta_{1-42}/A\beta_{1-40}$. No differences were detected in plasma neurogranin between controls and AD. Also, there was no correlation between CSF and plasma neurogranin, excluding confounding effects of the latter.

Conclusions: This study strengthens the potential of neurogranin as an AD CSF biomarker, which now needs validation in larger studies. As tools, straightforward immunoassays can be used, as demonstrated by the described ELISA.

Keywords: Neurogranin; CSF biomarker; Plasma biomarker; Prognostic biomarker; Alzheimer's disease; Mild cognitive impairment; tau; Amyloid; γ-secretase; ELISA; Ratio amyloid β

A.D.V. and D.J. are employees and shareholders of ADx NeuroSciences NV, co-developers of kits made commercially available by EUROIMMUN, Germany. K.B. has served at Advisory Boards for Eli Lilly, Pfizer, Roche, and Kyowa Kirin Pharma. S.E. served at Advisory Boards for Janssen, Pfizer, Roche, Innogenetics/Fujirebio Europe, Nutricia/Danone, and Novartis. E.V. is co-founder of ADx NeuroSciences NV. All other co-authors have nothing to disclose.

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http://dx.doi.org/10.1016/j.jalz.2015.05.012

1. Background

There is a need for improved early diagnosis of Alzheimer's disease (AD), especially in stages before dementia onset. Current parameters, such as the cerebrospinal fluid (CSF) biomarkers tau and $A\beta_{1–42}$, are highly predictive for the imminent occurrence of AD but have poor prognostic value regarding onset of cognitive decline [1]. From a neuropathologic perspective, the link between cognitive decline and tau/tangle or amyloid/plaque pathology seems complex [2]. Because the loss of brain synapses is much closer related to cognitive decline [3,4], a synaptic CSF biomarker could thus improve the prognostic accuracy of the AD biomarker panel.

Gained insights on neurogranin suggest that this postsynaptic protein could be a surrogate biomarker for synaptic loss. Neurogranin is important for long-term potentiation and learning abilities, mostly through interaction with calmodulin [5], and is found in CSF under physiological conditions [6–8]. Interestingly, in AD, neurogranin levels are decreased in the brain [9,10], whereas CSF levels are elevated as shown in our pilot study using immunoprecipitation and semiquantitative immunoblotting [11]. Recently, the AD-related increase in CSF neurogranin levels, which were moreover correlated with CSF tau concentrations, was verified by an enzyme-linked immunosorbent assay (ELISA) based on a new monoclonal antibody (mAb). Furthermore, the elevated neurogranin levels correlated with cognitive decline in prodromal AD [12]. These findings clearly support the potential of neurogranin as an AD CSF biomarker and warrants further studies. Finally, there is emerging evidence that neurogranin is also circulating in blood in the ng/mL range [13,14]. However, whether neurogranin could also be considered as an AD plasma biomarker has not been investigated up to date.

Accordingly, straightforward, yet highly specific, robust tools are required for further studies, allowing accurate measurements of neurogranin. In detail, hybrid immunoaffinity-mass spectrometry revealed CSF neurogranin as a pool of predominantly C-terminal peptides, whereas the full-length sequence was only present in minor quantities [12]. Targeting specific metabolic fragments is suggested to improve diagnostic performance of immunoassays, as demonstrated for tau of which predominantly N-terminal and mid-domain fragments are distinguished in CSF [15]. Therefore, we aimed to develop an ELISA during this study specific for the C-terminal part of neurogranin. Additionally, using this new prototype assay, we assessed neurogranin as a biomarker in CSF and plasma during an explorative study in paired CSF/plasma samples, collected from participants covering the whole disease spectrum of AD, from cognitively normal to experiencing full-blown dementia. The relationship between neurogranin and available clinical parameters, e.g. Mini-Mental State Examination (MMSE), was considered during the analysis as well as the link with the classical AD CSF biomarkers, e.g. CSF tau, but also plasma tau. Recent work demonstrated no correlation between tau levels in CSF and plasma [16]. By selecting a subgroup of patients with very high CSF tau levels, we wanted to confirm this observation and extend it by also investigating the relationship between neurogranin and tau in CSF/plasma. Finally, because the ratio CSF $A\beta_{1–42}$/CSF $A\beta_{1–40}$ appears to perform diagnostically better than the levels of the single analyte CSF $A\beta_{1–42}$ [17], we investigated the relationship between CSF neurogranin and CSF $A\beta_{1–42}$/CSF $A\beta_{1–40}$.

2. Methods

2.1. Study population

CSF/plasma samples from patients recruited in the Memory Clinic and Department of Neurology of Hospital Network Antwerp were selected from the Biobank of Institute Born-Bunge (Antwerp, Belgium). Patients with MCI due to AD (referred to as "MCI" hereafter) (n = 20), dementia due to AD ("AD") (n = 20), and patients with MCI or AD with high CSF T-tau levels ("high tau") (n = 20) were included. Diagnosis was based on the National Institute on Aging-Alzheimer's Association criteria [18,19], whereby CSF biomarkers $A\beta_{1–42}$, T-tau, and P-tau_{181P} were analyzed at the Institute Born-Bunge using commercial kits (INNOTEST β-$AMYLOID_{(1–42)}$, INNOTEST hTAU-Ag, and INNOTEST PHOSPHO-$TAU_{(181P)}$; Fujirebio Europe; Belgium) and whereby the institute's cutoff values were applied to discriminate AD from controls [20]. All patients had high probability of AD etiology except for one MCI patient who had intermediate probability. Also, one MCI patient carried a *PSEN1* mutation. The "high tau" group included MCI (n = 2) and AD (n = 18) patients demonstrating CSF T-tau levels >1200 pg/mL. Regarding the participants of the control group ("CTRL") (n = 30), cognitive deterioration was ruled out by neuropsychological screening, as well as neurologic or psychiatric antecedents, or central nervous system disorders. The study was approved by the local ethics committee (University of Antwerp). All subjects gave written informed consent.

CSF samples were obtained by lumbar puncture (LP) at the L3/L4 or L4/L5 interspace, collected in polypropylene tubes, and immediately frozen in liquid nitrogen. Plasma samples were collected immediately after LP, centrifuged at room temperature (RT) (10 min, 3000 rpm), transferred to polypropylene tubes, and immediately frozen in liquid nitrogen. Both types of samples were stored at −80°C until analysis.

From one-third of the participants, brain magnetic resonance imaging scans were available. The hippocampal volume (HCV) was visually rated by two radiologists, separately, blinded for diagnosis [21]. If needed, a consensus rate on divergent rates was discussed. On some participants *APOE* genotyping was performed. The single nucleotide polymorphisms in *APOE*—rs429358 and rs7412—determining the ε2/ε3/ε4 polymorphism were genotyped by