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ScienceDirect
Neuroscience Letters 427 (2007) 127–131
Neuroscience Letters
www.elsevier.com/locate/neulet

# The normal equilibrium between CSF and plasma amyloid beta levels is disrupted in Alzheimer's disease

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Received 9 June 2007; received in revised form 23 August 2007; accepted 12 September 2007

**Abstract**

Amyloid-beta (Aβ) with 40 (Aβ40) and 42 (Aβ42) amino acids, the main components of amyloid plaques in the Alzheimer's disease (AD) brain, can be measured in human cerebrospinal fluid (CSF) and plasma. Whereas CSF Aβ42 is decreased in AD, some studies have reported changed plasma Aβ levels in AD and in subjects with mild cognitive impairment (MCI). To this date it is unclear if and how CSF and plasma levels of Aβ correlate with each other in healthy individuals, albeit earlier studies on AD patients found no correlation between CSF and plasma Aβ. We have measured Aβ40 and Aβ42 in paired CSF and plasma samples from patients with AD ($n = 39$), MCI ($n = 29$) and healthy control subjects ($n = 18$). We observed a clear correlation between CSF and plasma levels for both Aβ40 and Aβ42 in healthy individuals, whereas no such correlation could be seen for AD or MCI cases. Similarly to other studies we also found low levels of Aβ42 in AD CSF, whereas there were no significant differences in plasma Aβ levels between the diagnostic groups. Our findings suggest that the normal equilibrium between CSF and plasma Aβ may be disrupted with the initiation of amyloid deposition in the brain.


*Keywords:* Alzheimer's disease; Mild cognitive impairment (MCI); Cerebrospinal fluid; Case control studies; Amyloid beta; APOE

Amyloid beta (Aβ) is the main constituent of plaques in the Alzheimer's disease (AD) brain. Apart from its presence in extracellular aggregates, soluble Aβ with either 40 (Aβ40) or 42 (Aβ42) amino acids can be found in intra- and extraneuronal compartments of different brain regions as well as in cerebrospinal fluid (CSF) and plasma.

Today, CSF Aβ42 measurement is a routine diagnostic procedure with 75–95% sensitivity and specificity [3,13,16]. Meanwhile, studies of Aβ levels in plasma are rather contradictory. Two studies have reported higher Aβ42 plasma levels in subjects with mild cognitive impairment (MCI) as compared to controls [1,25], while yet another study found lower plasma levels of Aβ42 in AD as compared to controls [22]. The different outcome of these studies might be caused by differences in study design, such as variations in age and disease severity of included subjects. Nevertheless, all studies have shown a substantial overlap in plasma Aβ40 and Aβ42 levels between patients and controls, thus limiting the diagnostic usefulness of plasma Aβ assessment.

It is unclear if and how levels of Aβ in CSF and plasma relate to each other. Three studies have reported a lack of correlation between CSF and plasma Aβ levels in AD patients [17,20,26]. In addition, yet another study failed to demonstrate any such correlation both for AD patients and healthy individuals [19]. Finally, it was recently shown that levels of Aβ from AD brain also do not correlate with plasma Aβ levels [8].

Contrary to the findings on human subjects, animal studies have demonstrated a clear relationship between CSF and plasma Aβ. In a study on rat, radiolabeled Aβ40 was shown to be rapidly cleared from ventricular CSF to blood [11], hence demonstrating an equilibrium between CSF and plasma Aβ levels . In addition, a relationship between CSF and plasma Aβ could be demonstrated in different transgenic mouse models of AD [4,15].

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doi:10.1016/j.neulet.2007.09.023

Table 1
Demographic data, APOE ε4 frequencies, CSF and plasma Aβ levels

| | Control | MCI | AD |
|---|---|---|---|
| Number | 18 | 29 | 39 |
| Age (mean ± S.D.) | 65.9 ± 8.6 | 60.3 ± 8.3 | 65.9 ± 7.9 |
| Male/female | 5/13 | 16/13 | 22/17 |
| APOE ε4 frequency (%) | 0.278 ($n$ = 9) | 0.352 ($n$ = 27) | 0.449 ($n$ = 39) |
| CSF Aβ40 (pM, mean ± S.D.) | 1415.5 ± 482.0 | 1579.9 ± 692.1 | 1387.9 ± 518.6 |
| CSF Aβ42 (pM, mean ± S.D.) | 191.6 ± 75.5 | 201.2 ± 111.8 | 122.7 ± 56.5* |
| Plasma Aβ40 (pM, mean ± S.D.) | 64.8 ± 17.0 | 61.1 ± 16.3 | 60.2 ± 13.1 |
| Plasma Aβ42 (pM, mean ± S.D.) | 25.4 ± 27.6 | 20.6 ± 11.9 | 21.6 ± 19.1 |

* $p$ = 0.0003 in comparison with healthy controls.

Levels of Aβ40 and Aβ42 have been the main markers of treatment outcome in several clinical studies [2,24]. Analysis of plasma instead of CSF Aβ in clinical trials is highly desired because of its greater accessibility. However, to ensure a correct interpretation of the plasma Aβ analysis, an increased knowledge on the relationship between CSF and plasma Aβ levels is essential.

In this study we have analyzed Aβ40 and Aβ42 levels in paired CSF and plasma samples from AD patients, individuals with MCI and cognitively healthy controls.

Between 2001 and 2003, 39 AD patients, 29 MCI patients and 18 healthy volunteers were consecutively investigated at the Memory clinic, Karolinska University Hospital Huddinge (Table 1). All patients underwent a standardized investigative battery, including medical history, physical and neurological examination, laboratory tests, neuropsychometric evaluation, electroencephalography (EEG), single photon emission computed tomography (SPECT) and either brain magnetic resonance imaging (MRI) or X-ray computed tomography (CT). The overall cognitive status was assessed by the Clinical Dementia Rating (CDR) scale and Mini Mental State Examination (MMSE) scores on all patients and control subjects [5]. At this point all study individuals also underwent a lumbar puncture, upon which CSF was collected.

Diagnosis of probable AD was made according to the National Institute of Neurological and Communication Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria [18] and complying with the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV. To be defined as MCI, the patients had to be free of significant underlying medical, neurological, or psychiatric illness and meet the following criteria (as defined and employed at Karolinska University Hospital [27]): (i) subjective memory complaint; (ii) objective signs of decline in any cognitive domain; (iii) intact activities of daily living; (iv) the clinical features does not fulfil the DSM-IV/ICD-10 criteria for dementia. The healthy volunteers were mainly recruited among the spouses of the patients included in this study.

The ethical committee of the Karolinska University Hospital and Uppsala University had approved the study.

Cerebrospinal fluid was obtained by lumbar puncture in the intervertebral space between the third and fourth or between the fourth and fifth lumbar vertebra and 12 ml was collected in polypropylene tubes. Within an hour, CSF samples were centrifuged at 3000 rpm for 10 min at 4 °C. The resulting supernatant was aliquoted in polypropylene tubes and stored at −80 °C until analysis. On the same occasion, heparin plasma samples were collected and stored at −80 °C.

All 39 AD patients, 27 MCI patients and 9 healthy individuals were genotyped for *APOE* by adopting a standard method [12].

Levels of Aβ40 and Aβ42 in plasma and CSF were assessed using a well-characterized ELISA [10]. This assay utilizes BNT77 (mouse IgA anti-Aβ 11-28; Takeda Pharmaceuticals, Osaka, Japan) as capture antibody and horseradish peroxidase-conjugated BA27 (IgG2 mouse anti-Aβ40) or BC05 (IgG1 mouse anti-Aβ42) as detector antibodies. In previous studies, adopting this ELISA, mainly EDTA plasma had been analyzed, but for our study only heparin plasma was available. Therefore, we initially compared Aβ40 and Aβ42 levels in several paired EDTA and heparin plasma samples. The results were found to be comparable for the two types of sample preparations (data not shown), which confirmed that heparin plasma was appropriate for Aβ analysis by this ELISA.

All plasma and CSF samples, which previously had been thawed once, were analyzed in triplicate with plasma and CSF from the same individual analyzed in parallel on the same ELISA plate. The researcher performing the analysis was blinded for the affection status of the individuals.

The Aβ levels in the different diagnostic groups were compared using Mann–Whitney $U$-test. Spearman Rank Order analysis was used to evaluate correlations. Correlations with $p$-value <0.05 were considered significant. No corrections for multiple comparisons were made. Subgroup analysis was used to evaluate gender influence.

A significant correlation between plasma and CSF levels of both Aβ40 (Spearman $r$ = 0.52) and Aβ42 (Spearman $r$ = 0.60) was found in healthy individuals, while no such correlation was observed in AD patients or subjects with MCI (Fig. 1). Moreover, in healthy individuals there was a clear correlation also between CSF Aβ40 and plasma Aβ42 (Spearman $r$ = 0.63). Since there was a gender discrepancy in healthy individuals when compared to AD and MCI patients, we also examined if the CSF–plasma correlation observed was gender dependent. However, no gender differences could be found.

Aβ42 levels in CSF of patients with AD were significantly decreased in comparison with healthy individuals and patients with MCI ($p$ < 0.001). Also the CSF Aβ42/Aβ40 ratio was significantly decreased in AD patients, mainly due to the decrease