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# Alzheimer's disease: Elevated pigment epithelium-derived factor in the cerebrospinal fluid is mostly of systemic origin

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ABSTRACT

Pigment-epithelium derived factor (PEDF) is a neurotrophic factor with neuroprotective, anti-tumorigenic, and anti-angiogenic effects. Elevated levels of PEDF have previously been proposed as a cerebrospinal fluid (CSF) biomarker for Alzheimer's disease. However, the origin of PEDF in CSF, i.e. whether it is derived from the brain or from the systemic circulation, and the specificity of this finding hitherto remained unclear. Here, we analyzed levels of PEDF in paired CSF and serum samples by ELISA in patients with Alzheimer's disease (AD, n = 12), frontotemporal dementia (FTD, n = 6), vascular dementia (n = 4), bacterial meningitis (n = 8), multiple sclerosis (n = 32), pseudotumor cerebri (n = 36), and diverse non-inflammatory neurological diseases (n = 19). We established CSF/serum quotient diagrams to determine the fraction of intrathecally synthesized PEDF in CSF. We found that PEDF is significantly increased in CSF of patients with AD, FTD, and bacterial meningitis. Remarkably, PEDF concentrations were also significantly elevated in serum of patients with AD. CSF/serum quotient diagrams demonstrated that elevated PEDF concentrations in CSF of patients with AD are mostly due to elevated PEDF concentrations in serum. These findings underscore the importance of relating concentrations of proteins in CSF to their respective concentrations in serum to avoid erroneous interpretations of increased protein concentrations in lumbar CSF.



## 1. Introduction

Alzheimer's disease (AD) is the most prevalent dementia in the elderly. Currently, AD is diagnosed only after a substantial number of brain cells have died or are irreversibly damaged [1,2]. With upcoming treatments aiming at preventing neuronal cell loss at an early disease stage [3], there is a need for early biomarkers of AD.

Pigment epithelium-derived factor (PEDF) is a 50-kDa neurotrophic factor shown to have neuroprotective, antitumorigenic, and antiangiogenic effects [4]. It was first discovered in conditioned medium from fetal human retinal pigment epithelial cells [5]. Nevertheless, it has meanwhile turned out that most mammalian tissues, including the brain, produce PEDF [6–9].

PEDF has been involved in several neurological disorders. In culture, it prevents cellular demise after glutamate- and 6-hydroxydopamine-induced neuronal toxicity [10–12]. Experimental cerebral ischemia in the rat leads to a downregulation of PEDF protein and mRNA between 1 and 3 days after the insult [13], whereas PEDF mRNA expression was increased between 1 and 7 days following experimental traumatic brain injury [14]. Application of PEDF either by gene transfer or osmotic pumps decreases disease severity after experimental cerebral ischemia [15] and traumatic brain injury [14], respectively.

PEDF in the CSF has previously been suggested as a biomarker for AD [16]. However, previous studies reported mixed results showing either an increase in PEDF concentration [16,17] or no change as compared to control patients [18,19]. To date, it thus remains unclear whether or

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not PEDF concentrations are elevated in the CSF of patients with AD. Furthermore, it is unknown whether PEDF in the CSF is produced intrathecally, e.g. by ependymal cells, or reaches the CSF from the systemic circulation.

In CSF/serum quotient diagrams, the CSF/serum quotient of the levels of a specific protein is plotted against the CSF/serum quotient of the levels of albumin ($Q_{Alb}$), a marker of the blood CSF barrier (BCSFB) function. Such diagrams permit to analyze the origin of a specific protein in the CSF, i.e. whether it is intrathecally synthesized or derived from the systemic circulation [20].

Based on previous data suggesting the PEDF levels are elevated in CSF of patients with AD, the aim of this study was to analyze whether elevated levels of PEDF in CSF originate from the CNS (intrathecal production) or the systemic circulation. In a second step, we wanted to compare the origin of CSF in different neuroinflammatory conditions to learn about the source of PEDF in CSF.

## 2. Material and methods

### 2.1. Patients

Lumbar punctures were performed with the patients' written informed consent for diagnostic purposes only. CSF and serum samples were collected between 2004 and 2010 at the Departments of Neurology, Charité - Universitätsmedizin Berlin, University Hospital Heidelberg, University Hospital Hamburg-Eppendorf and the NeuroCure Clinical Research Center in Berlin and stored at −80 °C. Only patients over 18 years of age were included in this study. To avoid misinterpretation of results due to artificial blood contamination of CSF samples, we included only CSF samples with an erythrocyte count ≤ 500/μl in this study [21]. Some of the patients included in this work were treated with different medications at the time of lumbar puncture.

#### 2.1.1. Non-inflammatory neurological diseases

Patients with non-inflammatory neurological diseases (NIN) included patients with tension headache (n = 3 patients) and epilepsy (n = 16). Tension headache was diagnosed according to standard clinical diagnostic criteria of the International Headache Society (ICHD-II) [22]. Epilepsy diagnosis was based on clinical findings and EEG studies. The group included patients with simple and complex focal seizures, primary and secondary generalized tonic-clonic seizures, or status epilepticus.

To establish the upper reference range for PEDF in the CSF/serum quotient diagrams, we included patients with non-inflammatory polyneuropathies (n = 7) and elevated CSF/serum albumin quotients. The diagnosis of peripheral polyneuropathy was based on the patients' history and clinical presentation, including typical findings in electrodiagnostic studies.

Patients included in the NIN group did not show any evidence of CNS inflammation in CSF as defined by (1.) a normal CSF lymphocyte count (≤5 cells/μl) and (2.) absence of intrathecal immunoglobulin (Ig) production (elevated IgG CSF/serum ratio [QIgG] or CSF-restricted oligoclonal Ig bands). Patients with NIN are herein referred to as “controls”.

#### 2.1.2. Dementias

Twenty-two dementia patients were included: 12 AD patients, six patients with frontotemporal dementia (FTD), and four patients with vascular dementia. Patients were diagnosed according to ICD-10 and the National Institute of Neurological and Communicative Disorders and the Stroke-Alzheimer's Disease and Related Disorders Association criteria (NINCDS-ADRDA 2004) [23].

#### 2.1.3. Bacterial meningitis

Eight patients with bacterial meningitis were included. Diagnosis was based on typical clinical presentation, e.g. headache, fever, meningism, elevated cell count, low glucose levels, increased lactate levels and BCSFB disruption with increased protein levels.

#### 2.1.4. Multiple sclerosis (MS)

This group included 32 patients with either relapsing-remitting MS (n = 25) or a clinically isolated syndrome suggestive of MS (n = 7). Diagnosis of MS was based on McDonald criteria [24]. Seventeen patients (53%) were in relapse at the time of lumbar puncture. All patients with MS showed intrathecal production of IgG.

#### 2.1.5. Pseudotumor cerebri (PTC)

Thirty-six patients with PTC were included. Diagnosis was based on clinical presentation and an increased CSF opening pressure (≥25 cm/$H_2O$ column) at lumbar puncture. Patients with PTC included in this work had no intrathecal Ig production or oligoclonal Ig in CSF, no BCSFB disruption, and a normal CSF cell count.

### 2.2. PEDF in CSF and serum

Serum and CSF concentrations of PEDF were measured by ELISA (BioProducts MD, LLC, USA) according to the manufacturer's protocol using a Tecan infinite M200 ELISA reader (Magellan™; Tecan, Crailsheim, Germany). 50 μl of CSF or serum were pretreated with 8 M urea. CSF samples were further diluted to a final concentration of 1/5000 and serum samples to 1/40,000 in assay dilutant.

### 2.3. IgG and albumin in CSF and serum

Total IgG and albumin concentrations in CSF and serum were measured nephelometrically (BN ProSpec, Siemens Healthcare Diagnostics, Marburg, Germany). As $Q_{Alb}$ is age-dependent, we calculated, for every patient, the age-dependent upper reference limit for $Q_{Alb}$ as described previously using the formula: $(\text{age [years]} / 15 + 4) \times 10^{-3}$ [25].

### 2.4. Statistical analysis

We performed all statistical analyses with SPSS v.19.0. We tested normality by Kolmogorov-Smirnov test. Variance homogeneity was evaluated using Levené test. When data were not normally distributed and variances were not homogenous across groups, as in the case of PEDF in the CSF (Kolmogorov-Smirnov test, $Z = 1.655$, $p = 0.008$, Levené test, $F(6,110) = 9.280$, $p < 0.001$), Kruskal Wallis test was performed followed by post hoc Mann-Whitney *U* test with α-correction according to Bonferroni to adjust for the inflation of type I error due to multiple testing (family-wise error rate). Here, we used corrected $\alpha = 0.5/k$ with $k = 9$ because we compared all patient groups to NIN (6 comparisons) as well as the different dementia groups with each other (3 comparisons), thus $p < 0.0063$ (= 0.05 / 9) was considered significant. Data are represented as medians. When data were normally distributed, as in the case of PEDF in serum (Kolmogorov-Smirnov test, $Z = 0.805$, $p = 0.536$, Levené test, $F(6,110) = 1.049$, $p = 0.398$), one-way ANOVA followed by Bonferroni post hoc was performed; $p < 0.05$ was considered statistically significant. Data are represented as mean ± standard deviation. For correlation analysis, we performed Pearson correlation for normally distributed data with $p < 0.05$ for statistical significance.

## 3. Results

A summary of the patients' demographic data is presented in Table 1. We analyzed PEDF concentrations in CSF and serum of the different patients groups compared to NIN patients. Table 1 shows a summary of the medians for PEDF concentrations in the CSF and means ± SD for PEDF concentrations in serum.