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## Correlations Between Cerebrospinal Fluid Biomarkers Concentration and Severity of Cognitive Impairment in Alzheimer Disease

### Korelacje pomiędzy stężeniami biomarkerów w płynie mózgowo-rdzeniowym a nasileniem zaburzeń funkcji poznawczych w chorobie Alzheimera

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#### Abstract

**Introduction.** Alzheimer's disease (AD) is a progressive, neurodegenerative disease and the most common cause of dementia.

**Aim.** The aim of the study was to assess the concentration of the 42 amino acid isoform of A $\beta$  (A $\beta$ 1-42), A $\beta$ 1-42/A $\beta$ 1-40 (42 amino acid isoform of A $\beta$ /40 amino acid isoform of A $\beta$ ) ratio, Tau and hyperphosphorylated Tau (pTau) protein in cerebrospinal fluid (CSF) of patients diagnosed with Alzheimer disease (AD) and to compare their correlations with degree of cognitive impairment assessed with Mini Mental State Examination (MMSE).

**Material and Methods.** In this study, using the ELISA immunoassay standard kits, we measured the average concentration of A $\beta$ 1-42, A $\beta$ 1-42/A $\beta$ 1-40 ratio, Tau and pTau protein, in the CSF obtained from subjects diagnosed with Alzheimer's disease (n=20, 13 women and 7 men, mean age 69.9 $\pm$ 10.4). The cognitive functions of the patients were assessed with MMSE test. The correlations between concentration of CSF biomarkers and degree of cognitive impairment were measured using nonparametric Spearman rank correlation coefficient.

**Results.** Our results showed negative correlation between concentration of Tau protein in CSF and the number of points scored in MMSE test (r=-0.45; p=0.046). There was no correlation between a degree of cognitive impairment assessed with MMSE test and concentration of pTau (r=-0.42; p=0.066), A $\beta$ 1-42 (r=0.02; p=0.927), and A $\beta$ 1-42/A $\beta$ 1-40 ratio (r=-0.07; p=0.775). There was also positive correlation between concentration of A $\beta$ 1-42 and A $\beta$ 1-42/A $\beta$ 1-40 ratio (r=0.91; p<0.0001), and between concentration of Tau and pTau (r=0.94; p<0.0001).

**Conclusions.** Tau protein plays not only a crucial role in the early diagnostics, but also reflects the intensity of cognitive impairment in course of Alzheimer disease. (JNPN 2018;7(4):150–154)

**Key Words:** Tau, pTau, Alzheimer's disease, Mini Mental State Examination, cognitive impairment

#### Streszczenie

**Wstęp.** Choroba Alzheimera (AD) jest postępującą chorobą neurodegeneracyjną i najczęstszą przyczyną otępienia.

**Cel.** Celem pracy była ocena stężenia 42 aminokwasowego A $\beta$ 1 (A $\beta$ 1-42), współczynnika 42 aminokwasowego A $\beta$ 1/40 aminokwasowego A $\beta$ 1 (A $\beta$ 1-42/A $\beta$ 1-40), białka Tau i nadmiernie ufosforylowanej formy białka Tau (pTau) w płynie mózgowo-rdzeniowym (PMR) pacjentów z rozpoznaną chorobą Alzheimera (AD) oraz porównanie ich stężenia ze stopniem zaburzeń funkcji poznawczych ocenianych przy pomocy krótkiej skali oceny stanu psychicznego (MMSE).

**Materiał i metody.** W badaniu, przy użyciu standardowych zestawów testów immunologicznych ELISA, oznaczono średnie stężenie A $\beta$ 1-42, współczynnika A $\beta$ 1-42/A $\beta$ 1-40, białka Tau i pTau w PMR pobranym od pacjentów z rozpoznaną chorobą Alzheimera (n=20, 13 kobiet i 7 mężczyzn, średnia wieku 69,9 $\pm$ 10,4 lat). Funkcje poznawcze pacjentów oceniano przy pomocy testu MMSE. Korelacje pomiędzy stężeniem biomarkerów w PMR a stopniem upośledzenia funkcji poznawczych były mierzone za pomocą nieparametrycznego współczynnika Spearmana.

**Wyniki.** Otrzymane wyniki wykazały negatywną korelację pomiędzy stężeniem białka Tau w PMR oraz liczbą punktów uzyskanych w skali MMSE (r=-0,45; p=0,046). Nie stwierdzono korelacji pomiędzy stopniem nasilenia zaburzeń

funkcji poznawczych ocenianych w teście MMSE a stężeniem pTau ( $r=-0,42$ ;  $p=0,066$ ), A $\beta$ 1-42 ( $r=0,02$ ;  $p=0,927$ ) oraz współczynnika A $\beta$ 1-42/A $\beta$ 1-40 ( $r=-0,07$ ;  $p=0,775$ ). Stwierdzono również pozytywną korelację pomiędzy stężeniem A $\beta$ 1-42 i współczynnikiem A $\beta$ 1-42/A $\beta$ 1-40 ( $r=0,91$ ;  $p<0,0001$ ) oraz pomiędzy białkiem Tau i pTau ( $r=0,94$ ;  $p<0,0001$ ).

**Wnioski.** Białko Tau odgrywa nie tylko kluczową rolę we wczesnej diagnostyce, ale również odzwierciedla nasilenie zaburzeń funkcji poznawczych w przebiegu choroby Alzheimera. (PNN 2018;7(4):150–154)

**Słowa kluczowe:** Tau, pTau, choroba Alzheimera, krótka skala oceny stanu psychicznego, zaburzenia poznawcze

## Introduction

Alzheimer's disease (AD) is a progressive, neurodegenerative disease, the most common cause of dementia. It accounts for about 5–11% of people aged over 65 years and 50% among those aged over 85 years. In AD pathophysiology there is intracellular accumulation of Tau protein (Tau) and hyperphosphorylated Tau (pTau) protein leading to formation of neurofibrillary tangles (NFTs) and extracellular accumulation of amyloid  $\beta$  (A $\beta$ ) in senile plaques [1,2].

Cerebrospinal fluid (CSF) biomarkers, such as Tau and pTau protein provide evidence of an ongoing AD pathophysiological process and may be good markers of cognitive impairment [2]. So far, some previous studies have reported an increased CSF concentration of Tau protein [3–5] and pTau [4,6], whereas some have found no significant change in Tau [6–10] and pTau [3,9–11] and one study reported a decrease of pTau concentration [8] in AD. Furthermore, CSF biomarker concentration have not correlated with cognitive impairment assessed with MMSE [7,12,13].

The Tau and pTau concentration correspond with the intensity of neuroaxonal degeneration. The concentration of pTau, correlates with tangle pathology, and the 42 amino acid isoform of A $\beta$  (A $\beta$ 42) concentration correlates inversely with amount of senile plaques in the brain [14]. It is well known that the pathological processes of neurodegeneration in the brain of AD patients start more than a decade before the first symptoms of cognitive impairment are noticed [15].

The aim of the study was to measure the average concentration of A $\beta$ 1-42, A $\beta$ 1-42/A $\beta$ 1-40 ratio, Tau, pTau proteins in CSF patients diagnosed with AD, and to compare their correlations with degree of cognitive impairment assessed with Mini Mental State Examination (MMSE).

## Materials and Methods

### Patients

The CSF samples were obtained from 20 patients (13 women and 7 men, aged  $69.9\pm 10.4$ ) admitted to the Department of Neurology, Medical University of Białystok. The study was approved by the Medical University of Białystok Ethics Committee for Research on Humans and Animals (R-I-002/382/2012) and written consent was obtained from all subjects. All individuals underwent lumbar puncture for diagnostic purposes.

The diagnosis of AD was based on NINCDS-ADRDA criteria (National Institute of Neurologic, Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association) [16]. All patients had progressive, significant episodic memory and impaired cognitive function lasting more than six months and validated using psychological tests.

### Samples Preparation and Evaluation Test

In patients with clinically probable AD we measured in the CSF the average concentration of A $\beta$ 1-42, A $\beta$ 1-42/A $\beta$ 1-40 ratio, Tau and pTau proteins using the ELISA INNOTEST® kits (Innogenetics GmbH, Hannover, Germany). The Cut-off values were provided by laboratory kit instruction. We also assessed patients' degree of cognitive impairment using Mini Mental State Examination (MMSE). MMSE is a 30-point questionnaire which assesses registration, attention, calculation, recall, language, repetition, ability to follow commands and orientation to time, place [17,18]. A score of  $\leq 9$  points indicates severe cognitive impairment, 10–18 points — moderate or 19–23 points — mild [17]. The characteristics of patients is shown in Table.

Table. The clinical characteristics of patients with Alzheimer disease

Number of patients (women)	Year	MMSE	A $\beta$ 1-42 (Cut-off=590 pg/mL)	Tau (Cut-off=300 pg/mL)	pTau (Cut-off=50 pg/mL)	Ratio A $\beta$ 1-42/A $\beta$ 1-40 (Cut-off=0.030)
20 (13)	$69.9\pm 10.4$	$20.3\pm 7.6$	$466.45\pm 296.65$	$353.35\pm 176.13$	$55.2\pm 21.67$	$0.025\pm 0.014$

MMSE — Mini Mental State Examination; A $\beta$ 1-40 —  $\beta$ -amyloid containing 40 amino acids; A $\beta$ 1-42 —  $\beta$ -amyloid containing 42 amino acids; Tau — Tau protein; pTau — hyperphosphorylated Tau protein

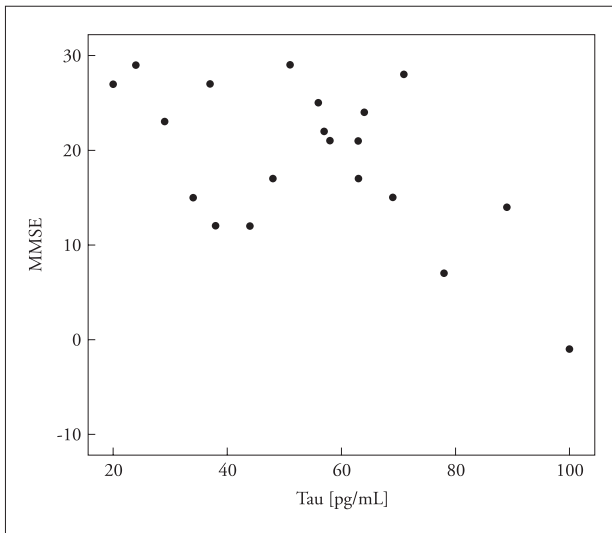


Figure 1. Correlation between concentration of Tau protein and the number of points scored in MMSE ( $r=-0.45$ ;  $p=0.046$ )

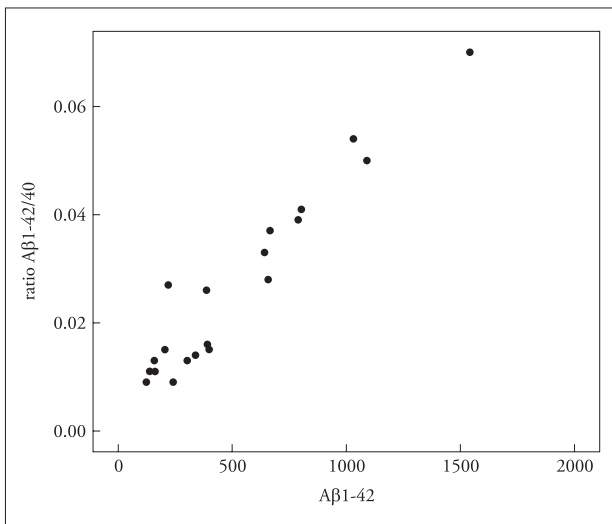


Figure 2. Correlation between concentration of Aβ1-42 and Aβ1-42/Aβ1-40 ratio ( $r=0.91$ ;  $p<0.0001$ )

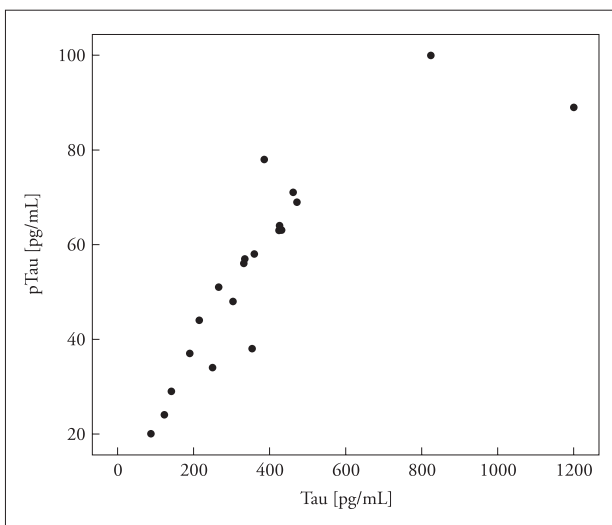


Figure 3. Correlation between concentration of Tau and pTau ( $r=0.94$ ;  $p<0.0001$ )

### Statistical Analysis

The statistical analyses were performed using the Statistica 10.0 program (StatSoft, Tulsa, OK, USA). Associations between variables were measured using the nonparametric Spearman correlation coefficient. P-value  $<0.05$  was considered as statistically significant.

### Results

The concentration of Tau protein in CSF of AD patients was  $353.35 \pm 176.13$  pg/mL, pTau  $55.2 \pm 21.67$  pg/mL, Aβ1-42  $466.45 \pm 296.65$  pg/mL, Aβ1-42/Aβ1-40 ratio  $0.251 \pm 0.014$  and the number of points scored in MMSE was  $20.3 \pm 7.6$  (Table).

Our results showed negative correlation between concentration of Tau protein in CSF and the number of points scored in MMSE test ( $r=-0.45$ ;  $p=0.046$ ) (Figure 1). There was no correlation between a degree of cognitive impairment assessed with MMSE test and concentration of pTau ( $r=-0.42$ ;  $p=0.066$ ), Aβ1-42 ( $r=0.02$ ;  $p=0.927$ ), and Aβ1-42/Aβ1-40 ratio ( $r=-0.07$ ;  $p=0.775$ ). There was also positive correlation between concentration of Aβ1-42 and Aβ1-42/Aβ1-40 ratio ( $r=0.91$ ;  $p<0.0001$ ) (Figure 2) and between concentration of Tau and pTau ( $r=0.94$ ;  $p<0.0001$ ) (Figure 3).

### Discussion

The pathological processes in the brain of AD patients start more than a decade before the first symptoms onset [15]. According to the cognitive impairment we may identify three stages of AD: preclinical AD, mild cognitive impairment (MCI) due to AD and AD with dementia [19]. The diagnosis of Alzheimer's disease (AD) is usually done in late stages of disease when patient presents advanced cognitive impairment [14]. The assessment of CSF biomarkers may establish AD diagnosis on an early stages of pathophysiological process before clinical symptoms of cognitive impairment.

The well known CSF biomarkers of AD are total amount of Tau protein, which reflects the intensity of neuroaxonal degeneration, pTau, which correlates with tangle pathology, and the 42 amino acid isoform of Aβ (Aβ42), which correlates inversely with number of senile plaques [14,20,21]. Tau is a microtubule-associated protein, located in the neuronal axons, responsible for microtubule assembly and stability [22]. In AD Tau becomes abnormally hyperphosphorylated, which is caused by an imbalance between multiple kinases and phosphatases [22]. Hyperphosphorylated Tau (pTau) forms intraneuronal filamentous inclusions called neurofibrillary tangles (NFTs) [23].

In our studies, we found that increased concentration of Tau protein in contrast to pTau, A $\beta$ 1-42, A $\beta$ 1-42/A $\beta$ 1-40 ratio in CSF patients with AD were associated with a higher degree of cognitive impairment measured with MMSE. This is in agreement with previous studies demonstrating that the concentration of Tau protein in CSF patients with AD positively corresponds with neuronal degeneration and cognitive decline [24]. Also in patients with mild cognitive impairment (MCI), high concentration of Tau and pTau proteins with low levels of A $\beta$ 1-42 are strongly associated with future development of AD [24–26].

In our study concentration of pTau protein did not correlate with cognitive impairment assessed in MMSE. Our patients were the elderly group (69.9 $\pm$ 10.4) in later stages of disease with MMSE (20.3 $\pm$ 7.6). This may result from that in later stages of AD there is decrease in pTau concentration, which probably is related to the extensive neuronal loss [27]. There are still conflicting data on pTau concentration in CSF of patients with AD. In some studies there were described a decrease of pTau concentration in AD [8] and an increase in stable MCI [27] and in some studies in AD patients [4,6]. The longitudinal changes in the CSF biomarkers did not correlate with cognitive functioning in one study [28], in another studies a decrease of pTau concentration correlated with faster MMSE decline [10,27], but in others an increase of pTau concentration correlated with cognitive impairment progression [10,13]. The increase of CSF Tau and pTau is rather seen in MCI and an early stages of AD, and the decrease in late stages of AD, which is associated with the extensive neuronal loss.

In accordance with previous studies, A $\beta$ 1-42 concentration and A $\beta$ 1-42/A $\beta$ 1-40 ratio did not correlate to the degree of cognitive impairment in our patients. It may be caused by the fact that A $\beta$ 1-42 deposition starts at an early stage of AD, when the brain atrophy is not so extended and cognitive impairment not severe yet [29,30].

## Conclusions

The results of our studies suggest that higher concentration of Tau protein in CSF patients suffering from AD indicates a crucial role of this protein not only in the early diagnostics, but also in assessment of the intensity of cognitive impairment in course of Alzheimer disease. Since neurodegeneration begins much earlier than the appearance of clinical signs, determining the appropriate diagnostic markers is important in the early diagnosis of Alzheimer disease.

## Implications for Nursing Practice

Alzheimer's disease (AD) affects mainly elderly people and significantly decreases patients and their families quality of life. Comprehensive approach in care over patient is recommended for nursery staff, including education of patient and his family on disease symptoms and teaching methods of coping with dementia.

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