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Usefulness of Cerebrospinalis Fluid Total Tau Concentration as Biomarker for Alzheimer's Disease*

Przydatność oceny stężenia całkowitego białka tau w płynie mózgowo-rdzeniowym jako biomarkera choroby Alzheimera

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Abstract

Background. Tau protein is a microtubule-associated protein in neuronal axons. Tau concentration in cerebrospinal fluid (CSF) has been used as a diagnostic marker for degenerative changes in the CNS. CSF/total tau is highly elevated in Alzheimer's disease (AD), but could be elevated in non-AD dementias as well.

Objectives. The aim of the study was to evaluate diagnostic accuracy of an AD test based on CSF/total tau concentration.

Material and Methods. A total of 102 persons were included in the study: 58 AD patients, 24 patients with vascular dementia (VD) and 20 nondemented controls. CSF samples were collected by routine lumbar puncture. The level of tau protein was determined by sensitive sandwich enzyme-linked immunosorbent assay (ELISA).

Results. CSF/total tau in the AD group (median 385.6 pg/ml) was higher than in VD group (median 101.2 pg/ml), and the controls (median 1.37 pg/ml). The differences were statistically significant. The cutoff value of 150 pg/ml distinguished AD from VD groups with sensitivity 79.2% and specificity 79.3%. Differences in tau protein concentration among the groups with AD, VD and control were attributable neither to the age nor sex of patients. Correlation between the Mini-Mental State Examination scores and CSF/total tau level in the AD group was high ($r = -0.66$) and statistically significant.

Conclusions. CSF/total tau concentration can be a valid diagnostic marker for AD (*Adv Clin Exp Med* 2005, 14, 3, 505–509).

Key words: tau protein, Alzheimer's disease, vascular dementia.

Streszczenie

Wprowadzenie. Białko tau, związane mikrotubulami komórkowymi, występuje w zakończeniach neuronalnych. Badanie stężenia w płynie mózgowo-rdzeniowym stosuje się do oceny zmian zwyrodnieniowych w o.u.n. Wykazano znacznie podwyższone stężenie tego białka w chorobie Alzheimera, może być również podwyższone w otępieniach niealzheimerowskich.

Cel pracy. Ocena diagnostycznej wartości stężenia całkowitego białka tau w płynie mózgowo-rdzeniowym w chorobie Alzheimera.

Materiał i metody. Badania przeprowadzono w grupie 102 osób, w tym u 58 chorych na chorobę Alzheimera oraz u 24 z rozpoznaniem otępienia naczyniowego. Dwadzieścia osób bez otępienia stanowiło grupę kontrolną. Płyn mózgowo-rdzeniowy pobierano za pomocą punkcji lędźwiowej. Badania stężenia białka tau przeprowadzono techniką ELISA.

Wyniki. Stężenie całkowitego białka tau w grupie pacjentów z rozpoznaną chorobą Alzheimera było większe (mediana 385,6 pg/ml) w grupie z otępieniem naczyniowym (mediana 101,2 pg/ml) i w grupie kontrolnej (mediana 1,37 pg/ml). Różnice były statystycznie istotne. Wartość odcięcia 150 pg/ml różnicowała chorobę Alzheimera od otępienia naczyniowego z czułością 79,2% i swoistością 79,3%. Różnic w stężeniach białka tau w poszczególnych

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grupach diagnostycznych (choroba Alzheimera, otępienie naczyniowe) i w grupie kontrolnej nie można było odnieść zarówno do wieku, jak i płci badanych. Korelacja między punkcją w skali MMSE a stężeniem całkowitego białka tau w płynie mózgowo-rdzeniowym w grupie pacjentów z chorobą Alzheimera była wysoka ($r = -0,66$) i statystycznie istotna.

Wnioski. Stężenie całkowitego białka tau w płynie mózgowo-rdzeniowym może być ważnym markerem diagnostycznym choroby Alzheimera (*Adv Clin Exp Med* 2005, 14, 3, 505–509).

Słowa kluczowe: białko tau, choroba Alzheimera, otępienie naczyniowe.

Tau protein (60–70 kD) is a microtubule-associated protein. It has tandem repeats of a tubulin binding domain, and promotes tubulin assembly. The tau proteins in fact constitute a family whose various members are made by alternative splicing of a single gene. They are predominantly associated with microtubules of the axon. Tau expression thus plays a role in the formation and maintenance of the integrity of the axonal structure. Aberrantly phosphorylated tau is also one of the major components of the paired helical filaments (PHF) that accumulate in neurones to form the neurofibrillary tangles, which with senile plaques constitute one of the neuropathological hallmarks of Alzheimer's disease (AD) [1–3]. There is in fact a strong correlation between the occurrence of neurofibrillary lesions and dementia, but the presence of such tangles can be determined in neuropathological analysis of specimens obtained from brain biopsy or post mortem [4]. Some authors have speculated that tau protein accumulates in the cerebrospinal fluid (CSF) of AD patients as a result of the progressive generation of tau and the massive death of neurones in the central nervous system (CNS) with the attendant spillage of the cellular contents into the matrix [5–7].

In the search for an adequate biochemical test allowing Alzheimer's disease to be diagnosed and its clinical course assessed without resorting to biopsy, it has been suggested that the tau protein level should be measured in the cerebrospinal fluid of AD patients in different stages of the disease [8–10]. At present, two biochemical markers, CSF-tau (total and phosphorylated) and CSF-beta amyloid (1–42) can be analysed to assess the progression of dementia, in addition to clinical anamnesis and brain imaging techniques. Various combinations have been tested, including not only the absolute values of total tau, phosphorylated tau, and A β 42, but also various proportions among them [11–13]. Of considerable importance from both the theoretical and clinical perspective is differentiation *in vivo* between AD, and other neurodegenerative diseases in older patients that can produce clinical signs of dementia, such as Lewy body dementia (LBD), frontotemporal dementia (FTD), vascular dementia (VD), and many other known clinical syndromes.

The aim of presented study was to evaluate diagnostic accuracy of an AD test based on CSF/total

tau concentration in determining differentiation Alzheimer's disease from vascular dementia.

Material and Methods

AD and VD patients were outpatients, recruited from members of the Lower Silesian Association of Alzheimer Patients' Families. The authors included subjects without dementia as controls, suffering from radicular disease, recruited from the Department of Neurology at the Wrocław Military Hospital. The clinical diagnosis of AD was based on the NINCDS-ADRDA criteria [14]. The clinical diagnosis of VD was made on the basis of NINCDS-AIREN criteria for vascular dementia [15]. Dementia severity was assessed and evaluated by means of the Mini-Mental State Examination (MMSE). The consent of the Regional Ethics Committee was obtained. All the patients or their relatives signed an informed consent prior to participation in the study.

Cerebrospinal fluid was collected from all patients by a routine lumbar puncture in the Department of Neurology at the Wrocław Military Hospital. The samples were centrifuged after collection at 1500 rpm for 10 minutes, then aliquoted and stored at -70°C until analysed. The level of tau protein was determined using the sensitive sandwich enzyme-linked immunosorbent assay (ELISA) technique developed by Vandermeeren et al. [16], with the use of a reagent kit based on nonspecific polyclonal antibodies made by Innogenetics (Belgium) according to the manufacturer's instructions. The assays of CSF-tau were performed in duplicate, with the differences between duplicate samples were under 10%.

Statistical differences in age and the CSF/total-tau concentration were analysed by means of the Mann-Whitney U test. Receiver operating characteristic (ROC) was analysed to assess the most appropriate cutoff values for the CSF/total-tau in the distinction between AD and VD groups.

Results

102 persons were included (Tab. 1). The dementia group consisted of 58 patients with suspect-

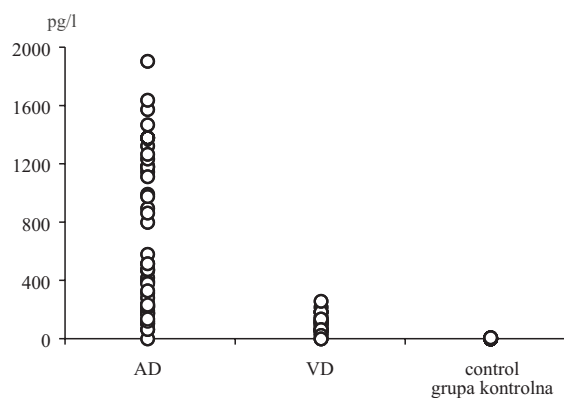
Table 1. Summary of the patient population**Tabela 1.** Dane demograficzne na temat populacji pacjentów

Group (Group)	Number – men/women (Liczba – kobiety/mężczyźni)	Age (Wiek)	MMSE
AD (Choroba Alzheimer)	58 (27/31)	68 ± 6.8	14.5 ± 6.1
VD (Otępienie naczyniowe)	24 (12/12)	65 ± 8.0	20.8 ± 3.4
No dementia (Brak otępienia)	20 (10/10)	64.5 ± 9.9	28.3 ± 1.1

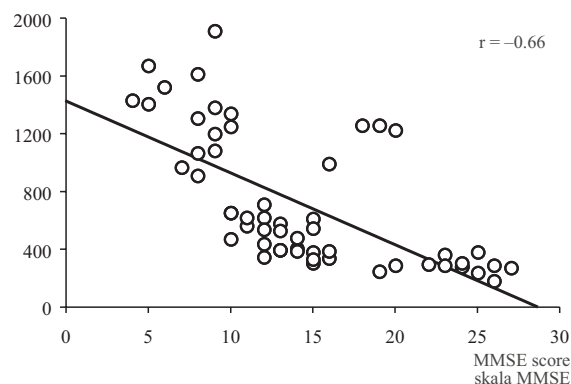
ed AD, and 24 VD patients. Control group consisted of 20 subjects without dementia.

Differences in number of males and females among groups with Alzheimer type dementia, vascular dementia and control were minor and negligible ($\chi^2 = 0.28$, $p = 0.60$). The groups differed slightly in mean age. Patients with AD (mean 68 years) were somewhat older than patients in VD group (mean 65 years), and persons in control group (mean 64.5 years), but differences were not significant (Mann-Whitney U test, $Z = 1.33$, $p = 0.18$ and $Z = 1.30$, $p = 0.20$ respectively). CSF/total-tau level in the group with Alzheimer type dementia (mean 678.0, median 385.6 pg/ml) was higher than concentration in both the VD group (mean 106.9, median 101.2 pg/ml), and the controls (mean 3.5, median 1.37 pg/ml) (Fig. 1). These differences were highly significant (Mann-Whitney U test, $Z = -5.36$, $p < 0.0001$ and $Z = 6.44$, $p < 0.0001$ respectively). The difference in CSF/total-tau between vascular dementia group and control group were also statistically significant (Mann-Whitney U test, $Z = -4.87$, $p < 0.0001$).

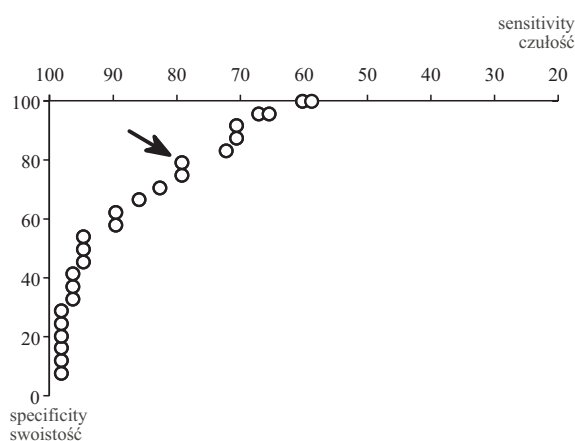
Correlation between the MMSE scores and CSF/tau-total level in the AD group was high and significant ($r = -0.66$, $p < 0.001$) (Fig. 2) but in the VD group was low and not significant ($r = -0.23$, $p < 0.28$). The age of patients had little influence on CSF/tau level. The product moment correlations between age and CSF/tau level showed a trend toward increasing with age but did not reach a significant level: in the group with AD $r = 0.24$, $p = 0.07$, in the group with VD $r = 0.12$, $p = 0.59$, and in the control group $r = 0.36$, $p = 0.12$. A gender has no statistically significant influence on CSF/total-tau in the AD group (Mann-Whitney U test, $Z = -0.44$, $p = 0.66$) nor in the VD group (Mann-Whitney U test, $Z = -0.35$, $p = 0.73$). Figure 3 shows ROC curve for

**Fig. 1.** CSF/total tau concentration in Alzheimer's disease, vascular dementia, and control

Ryc. 1. Stężenie całkowitego białka tau w płynie mózgowo-rdzeniowym w chorobie Alzheimera, otępieniu naczyniowym i w grupie kontrolnej

**Fig. 2.** Correlation between CSF/total-tau concentration and MMSE score in Alzheimer's dementia group

Ryc. 2. Zależność między stężeniem całkowitego białka tau w płynie mózgowo-rdzeniowym a punktacją MMSE w otępieniu typu Alzheimera

**Fig. 3.** Receiver operating characteristic (ROC) for the CSF/total-tau. An arrow shows an optimal cutoff 150 pg/ml with sensitivity of 79.2% and specificity 79.3%

Ryc. 3. Charakterystyka czułości/wrażliwości dla stężenia całkowitego białka tau w płynie mózgowo-rdzeniowym. Strzałka wskazuje na optymalną wartość odcięcia 150 pg/ml z czułością 79,2% i swoistością 79,3%

CSF/total-tau. The ROC curve demonstrates that cutoff value of 150 pg/ml most reliably distinguished AD from VD groups. This cutoff yielded a sensitivity of 79.2% and a specificity of 79.3%. The difference between AD and control groups was larger. The lowest CSF/total-tau level for AD group (65.2 pg/ml) was above the highest level for the control group (13.3 pg/ml). One negative measurement in AD group was considered as an artifact.

Discussion

The results of presented experiment support the hypothesis of a significantly higher concentration of tau protein in the cerebrospinal fluid of AD patients in comparison with VD patients and the controls. The increase in CSF/total-tau in AD has been demonstrated in a number of studies [1, 3–5,

9–11, 17]. The value of CSF/total-tau to discriminate between AD and other dementias is relatively high, with sensitivity and specificity about 80% [17, 23, 24]. The study yields similar values of these parameters. Some newer studies showed that CSF/phospho-tau slightly better discriminates AD. Levels of p-tau(231) discriminated with a sensitivity of 90.2% and a specificity of 80.0% between AD and all non-AD disorders. Moreover, p-tau(231) levels improved diagnostic accuracy compared with t-tau levels when patients with AD were compared with healthy controls demented subjects with frontotemporal dementia, but not those with vascular and Lewy body dementias [25]. Some findings suggest that total tau and phospho-tau in CSF reflect different pathogenic processes in the brain; total-tau the degree of neuronal damage and phospho-tau the phosphorylation state of tau and thus possibly the formation of neurofibrillary tangles [26].

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