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USE OF NEW MARKERS IN THE DIAGNOSTICS OF NEURODEGENERATIVE DISEASES

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A b s t r a c t

The authors report on the possibilities of the clinical use of tau protein, beta amyloid, and cystatin C as new markers in the diagnostics of neurodegenerative diseases (ND). In this study the levels of tau protein, beta amyloid, and cystatin C in the diagnostics of some of the above-mentioned diseases were assessed. We found statistically significantly increased levels of tau protein in 60.3 % of patients with ND (diagnosis (dg.) 1+2), and in 92 % of patients with Alzheimer's dementia (AD, dg. 2). With decreased levels of beta amyloid, the Mann-Whitney test showed statistically significant differences between ND and other diagnostic groups, but using a chi-square test there were no statistically significant differences between both groups. For the tau protein/beta amyloid index we found increased levels in 87.5 % patients with AD (dg. 2) using a chi-square test and also statistically significant differences compared to groups with non-degenerative diseases. The levels of cystatin C in the sera were decreased in the ND group statistically significantly compared to inflammatory diseases of the CNS. There was no statistically significant difference in cystatin C cerebrospinal fluid (CSF) levels for each diagnostic group. We found increased levels of the index of cystatin C (CSF C/serum C) in the group with ND, especially with dg. 2, which is statistically significant for groups with non-degenerative diseases. With tau protein our results satisfactorily prove a claim of this marker on its possible use in the diagnosis of AD, in the case of beta amyloid the result is at least questionable. The existence of decreased cystatin C levels in the sera and CSF in patients with ND presumed in earlier studies was not sufficiently confirmed on the basis of our study.

K e y w o r d s

Tau protein, Beta amyloid, Cystatin C, Biochemical diagnostics, Neurodegenerative diseases, Alzheimer's dementia

INTRODUCTION

At present, many authors turn their interest to new possibilities in the diagnosis of neurodegenerative diseases (ND). Support for the diagnosis of Alzheimer's dementia (AD) is now provided by cerebrospinal fluid assessment for the levels of beta amyloid and tau protein by the methods of ELISA and fluorescence correlation spectroscopy (FCS). Beta amyloid protein is a component of the amyloid plaque accumulating in the AD brain. Tau protein occurs in the cytoskeleton of

CNS neurons, where it stabilises the axonal system; there are low concentrations of tau protein in the serum and CSF of healthy individuals. Among other markers that can contribute to the diagnosis of ND, we can recently count cystatin C. It is an amyloid protein that occurs together with beta amyloid in the walls of arterioles in patients with AD. It is an inhibitor of cysteine protease, relatively stable in circulation, and is also known for its clinical utility as an indicator of glomerular filtration (GF) (*1*); cystatin C concentration is considered an excellent correlate of GF levels because, in contrast to creatinine and the clearance of endogenous creatinine, it is not affected by food, extra-glomerular secretion, the accuracy of urine collection, and analytical interferences (*2*). Cystatin C is usually assessed in the biological material by three techniques: the advantage of the ELISA method lies in the possibility of measuring low concentrations but there is no possibility of making a statim assessment. Statim testing is possible by nephelometry and turbidimetry, which in turn offer no possibility of low concentration measurement. Turbidimetry suffers the disadvantages of small robustness and low calibration stability. Nephelometry is currently the best method for cystatin C measurement.

In a subset of AD patients, a polymorphism of cystatin C gene (polymorphism of A gene for cystatin C is joined to higher risk of AD) and a decreased level of cystatin C in the serum were found (*3, 4*). Cystatin C also participates in the neuronal degenerative as well as reparative processes, it probably inhibits the activity of cathepsin L in astrocytes (which together with cathepsin S belongs to the key components of the immune potential of astrocytes and microglia) (*5*). It seems that patients with AD have very low concentrations of cystatin C in CSF and sera (cystatin C is accumulated in reactive astrocytes before amyloid formation), and that the disease is manifested by congenital haemorrhage and amyloidosis (*6*).

The biochemical markers of ND may contribute to diagnostics (tau protein, phosphorylated on serine 199 in CSF) and can be used to monitor treatment effects (cystatin C in CSF). In the case of AD, first the investigated use of tau protein and beta amyloid was to aid in the diagnosis of the disease. In patients with AD, decreased levels of beta amyloid and increased levels of tau protein were found. Their ratio – the tau/beta index – is usually increased and has an almost absolute sensitivity, high specificity, and also a high negative predictive value in the AD diagnosis.

The assessment of these markers can also contribute to the differential diagnosis of vascular dementia but also to distinguishing other forms of primary degenerative dementia, such as frontotemporal dementia. In the study of *Hulstaert et al.* (*7*), tau protein and beta amyloid CSF levels were examined in a set of 413 patients. In the subgroup of patients with AD (n=150), a significant increase of tau protein against normal levels was reported. In other groups (and also in healthy volunteers), such as patients with difficulties without pathological processes in the brain, patients with other neurological difficulties and patients with the non-AD type of dementia, there were no reported increases of tau protein. On the contrary, a substantial decrease

of beta amyloid was described in patients with AD compared to the other groups. According to these studies, both compounds are acceptable markers for early diagnosis of AD.

Assessment of tau protein and beta amyloid together demonstrated a predictive value for over 90 % for AD diagnosis and according to some studies it can also help in differential diagnosis of Pick dementia (FTD) because CSF tau protein levels in FTD patients are higher than in the control group but lower than in the AD group, whereas CSF beta amyloid levels are lower in FTD when compared to the control group but higher than in the AD group (*8*). A reduction of cystatin C levels was observed in patients with bacterial meningitis (*9*). According to some authors, cystatin C is involved in neurodegenerative and repair processes after ischaemic and mechanical injuries (*10, 11, 12*). Abnormally low cystatin C levels were described in the Icelandic variant of hereditary cerebral haemorrhage with amyloidosis (HCHWA), the hereditary form of cerebral amyloid angiopathy (CAA), and in progressive dementia (*13*).

In our prospective, bicentric, hospital-based study which has been realised at the Department of Neurology, University Hospital in Olomouc, and in Hospital Šternberk, we studied the significance of tau protein, beta amyloid and cystatin C levels in the diagnostics of some of the above-mentioned diseases and we tried to evaluate its contribution to differential diagnostics.

MATERIAL AND METHODS

A set of patients (n = 434, 212 males and 222 females, age 5–92, mean 62 $\pm$ 14, 73) at the Department of Neurology, University Hospital in Olomouc and Hospital Šternberk was divided into 9 subgroups to compare neurodegenerative diseases (groups 1+2) with the other diagnostics groups (groups 3–9): 1 – neurodegenerative diseases (m. Parkinson, spinocerebellar ataxia, heredodegenerative neuropathy, n = 43), 2 – Alzheimer's dementia (AD, n = 26), 3 – serous inflammation of CNS (n = 33), 4 – bacterial inflammation of CNS (n = 4), 5 – multiple sclerosis (MS, n = 71), 6 – demyelinisation neuropathy (AIDP = Acute Inflammatory Demyelinating Polyneuropathy, CIDP = Chronic Inflammatory Demyelinating Polyneuropathy), n = 15), 7 – ischaemic affection of CNS (n = 41), 8 – haemorrhagic stroke (n = 5), 9 – other diagnoses without CNS affection (n = 196).

The CSF was collected in all patients on the basis of informed consent. Basic morphological and biochemical assessment (specific proteins, haematoencephalic barrier integrity test, Reiber calculation) was performed. The ELISA method was used for the assessment of the particular markers: tau protein (ELISA, Biosource, UK), beta amyloid (ELISA, Innogenetics, Belgium), and cystatin C (ELISA, Biovendor, Brno, Czech Republic). The whole study was conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983) and was approved by the local ethics committee of our hospital.

The normal CSF reference values were: 0–210 ng/l for tau protein, 400–1200 pg/l for beta amyloid, 0–0.5 for tau/beta index, 3–12.5 mg/l for cystatin C. For serum cystatin C, the normal reference values were 0.63–1.44 mg/l.

RESULTS

A basic description of tau protein, beta amyloid, and cystatin C values in groups of neurodegenerative and non-degenerative diseases is reported in *Table 1*.

We found statistically significantly increased levels of tau protein in 60.3 % of patients with ND (dg. 1+2) and in 92 % of patients with AD (dg. 2) by the chi-square test compared to groups 5, 7, 8 (*Fig. 1*).

Table 1

Basic description of cystatin C, tau protein, and beta amyloid for both groups of neurodegenerative and non-degenerative diseases

Report

DG		CYS C CSF	CYS C S	CYS C CSF /CYS C S	BETA A	TAU	TAU / BETA A
1+2	N	52	32	32	43	59	43
	Minimum	2,05	,463	1,48	125,0	89	,096
	Maximum	25,00	1,380	30,80	2.003,0	1200	2,309
	Median	4,60	,759	7,67	649,6	283,00	,402
	Mean	6,55	,799	9,67	703,5	328,29	,607
	Std. Deviation	5,63	,221	6,92	409,4	240,10	,524
3-9	N	290	209	204	156	177	155
	Minimum	,75	,438	,54	217,1	24	,035
	Maximum	25,00	3,049	45,45	2.003,0	1200	5,527
	Median	3,96	,800	5,44	772,3	221,00	,264
	Mean	5,34	,867	7,56	812,1	257,28	,398
	Std. Deviation	4,31	,338	6,74	338,6	188,22	,569

In the case of beta amyloid, we found only a borderline trend (compared to other groups) to its decrease, in both groups with ND normal levels of beta amyloid were usually found. In the group of AD patients it was only 31.3 % but, with regard to the other diagnosis, it was the highest number. The Mann-Whitney test showed statistically significant differences between the levels of beta amyloid (*Fig. 2*) in subgroups 1+2 and subgroups 3–9, but in the chí-square test there were no statistically significant differences between both groups.

For the tau protein/beta amyloid index, we found increased levels in 44.2 % patients with ND (dg. 1+2). In patients with AD (dg. 2), it was 87.5 % patients – a statistically significant difference (chi-square test) compared to groups 3, 4, 5, 9 (*Fig. 3*).

The levels of cystatin C in the serum were decreased in the group with ND (dg. 1, 2), which is a statistically significant difference compared to subgroup 3. Higher levels of cystatin C were found in subgroup 8, which differs statistically significantly from group 9. There was no statistically significant difference in cystatin C CSF levels for each diagnostic group. The highest number of decreased levels is shown by the

group with inflammatory diseases of CNS (dg. 3, 4). We found increased levels of the cystatin C index (CSF C/serum C) in the group with ND, especially with dg. 2, which is statistically significant for dg. 3, 6, 7, and 9 (*Fig. 4*).

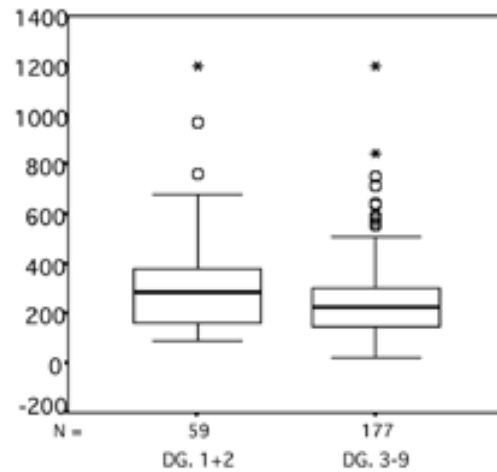

Fig. 1
Tau protein in neurodegenerative and non-degenerative diseases

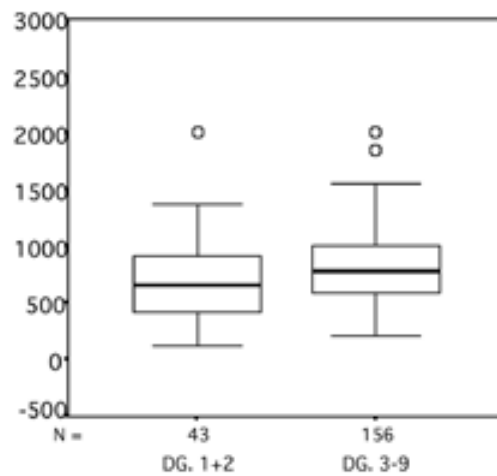

Fig. 2
Beta amyloid in neurodegenerative and non-degenerative diseases

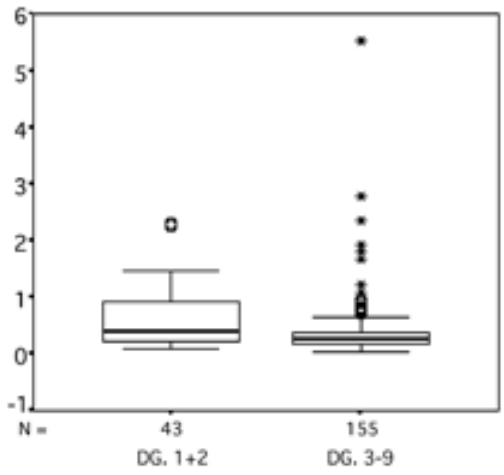

Fig. 3
Tau protein/beta amyloid index in neurodegenerative and non-degenerative diseases

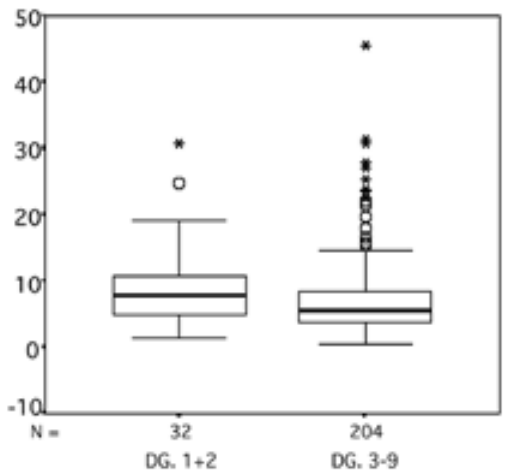

Fig. 4
Cystatin C index in neurodegenerative and non-degenerative diseases

DISCUSSION

Our findings of increased tau protein levels in CSF in patients with ND (dg. 1, 2) confirmed and replicated early literary data (*7,14,15*). Though this is only a borderline trend, we can basically confirm the published hypothesis about the decrease of CSF beta amyloid in AD patients (*16*).

According to the study of *Kálman* (*17*), there were no statistically significant differences between the cystatin C levels in the serum and CSF in patients with AD and ischaemic vascular dementia compared to the other groups of patients. *Shimode* (*18*) came to similar conclusions. In contrast, other studies (*3,4*) report decreased levels of cystatin C in the serum of patients with AD. In our study, 42.9 % of AD patients had decreased serum levels of cystatin C, which is the highest number from all diagnostic groups, but still the remaining 57.1 % of AD patients had normal levels. Cystatin C levels in the CSF of AD patients showed normal levels in 70.6 %; therefore we cannot confirm the suggestion of its decreased levels in this type of disease.

Decreased levels of cystatin C were described in patients with local and systemic inflammatory diseases (*19*). In our study, no cases of decreased levels of cystatin C were detected in the group of patients with serous inflammatory diseases of CNS (dg. 3, 4); on the contrary, this group included the highest percentage (16.7 %) of increased serum levels of cystatin C compared to the other diagnostic groups.

According to the study of *Nagai* (*20*), a significant decrease of cystatin C was observed in patients with the Guillain-Barré syndrome, chronic inflammatory demyelinisation polyneuropathy (CIDP) and RS compared to the control group. The levels of cystatin C may correlate with the high values of cathepsin B that are described in patients with these diseases. In our study, these groups manifested normal levels (in 100 % in dg. 6).

CONCLUSIONS

Our study confirms the previously observed results only partially – in the case of CSF tau protein, our data strongly support a claim of this marker for its possible use in the diagnosis of AD, whereas in the case of CSF beta amyloid, the result is at least questionable. The presence of decreased cystatin C levels in the serum and CSF in patients with ND suggested in earlier studies was not convincingly confirmed in our study.

A c k n o w l e d g e m e n t s

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VYUŽITÍ NOVÝCH MARKERŮ V DIAGNOSTICE NEURODEGENERATIVNÍCH ONEMOCNĚNÍ

S o u h r n

Autoři referují o možnostech klinického využití tau proteinu, beta amyloidu a cystatinu C jako nových markerů v diagnostice neurodegenerativních onemocnění (ND). V této studii byly porovnány hodnoty těchto ukazatelů u ND a dalších neurologických onemocnění. Byly nalezeny statisticky signifikantně zvýšené hodnoty tau proteinu u pacientů s ND – 60.3% u pacientů s Alzheimerovou demencí (AD) a jinými ND, přičemž u pacientů s AD byly hodnoty zvýšeny v 92%. U snížených hodnot beta amyloidu byly podle Mann-Whitney testu prokázány statisticky signifikantní rozdíly mezi skupinou pacientů s ND a pacientů s jiným neurologickým onemocněním, avšak chí-kvadrát test tyto rozdíly neprokázal. Pro index tau protein/beta amyloid byly nalezeny zvýšené hladiny u 87.5% pacientů s AD, chí-kvadrát test prokázal statisticky signifikantní rozdíl v porovnání se skupinou jiných neurologických onemocnění. Hodnoty cystatinu C v séru byly sníženy ve skupině s ND – statisticky signifikantně v porovnání se zánětlivými onemocněními CNS. Nebyly zjištěny statisticky signifikantní rozdíly u cystatinu C v mozkomíšním moku (CSF). Zvýšené hodnoty indexu cystatinu C (CSF C/serum C) byly nalezeny u pacientů s ND, zvláště pak u skupiny s AD, která se statisticky signifikantně lišila od skupiny s jinými neurologickými onemocněními. V případě tau proteinu naše výsledky uspokojivě podporují teorii o tau proteinu jako diagnostickém markeru AD, v případě beta amyloidu jsou výsledky přinejmenším diskutabilní. Existence snížených hodnot cystatinu C v séru a CSF u pacientů s ND, které prokázaly některé dřívější studie, nemůžeme na základě našich výsledků dostatečně potvrdit.

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