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Brief Report

# Decreased Concentration of Annexin V in Parkinsonian Cerebrospinal Fluid: Speculation on the Underlying Cause

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**Summary:** Circumstantial evidence suggests that increased apoptosis is responsible for the loss of dopaminergic nigrostriatal neurons in Parkinson's disease (PD). It is impossible to perform high-quality studies on human postmortem material because of the low quality of tissue preservation, and the fact that apoptosis has a duration of only hours, and that the duration of the agonal period itself will lead to massive neuronal cell death. We measured, as epiphenomenon of neuronal cell death ex vivo, the Annexin V concentration in cerebrospinal fluid (CSF) in patients with PD and control subjects. The Annexin V concentration in CSF of patients with PD was significantly lower compared with control subjects. Annexin V concentrations of the CSF did not correlate with dementia, duration of symptoms, age, sex, or treatment of PD. The rationale for measurement of Annexin V in CSF is the fact that Annexin V adheres to dying cells. It is tempting to suppose that the decrease of Annexin V in CSF of PD is the result of consumption of this protein during neuronal apoptosis as has been demonstrated to occur in the midbrain in PD. **Key Words:** Parkinson's disease—Cerebrospinal fluid—Annexin V—Apoptosis.

Apoptosis has been postulated to be the mechanism responsible for the loss of the dopaminergic nigrostriatal neurons in Parkinson's disease (PD).[1,2] Mochizuki et al.[3] reported evidence of apoptosis in substantia nigra neurons of autopsied patients with PD. Nick-end labeling histochemistry of midbrains showed intense nuclear staining in eight of 11 patients with PD who were studied. Tatton et al.[4] showed, with in situ end-labeling by using a sensitive fluorescent double-labeling technique in combination with a cyanide dye to demonstrate nuclear chromatin condensation, that dopaminergic neurons in the substantia nigra die through apoptosis in PD.

Magi et al.[5] found significantly higher soluble Fas (sFas) in dopaminergic nigrostriatal regions in patients with PD postmortem compared with control brains. High concentrations of sFas have been described in disorders with increased apoptosis.[6,7]

The chances to prove convincingly disease-related apoptosis in brains of autopsied patients with PD, who had this disease for several years, are extremely low considering the fact that apoptosis has a duration of some hours. In addition, the anoxia during the agonal period and certainly after death will lead to massive cell death, particularly in neurons. Therefore, the question of whether apoptosis plays a key role or is secondary to the occurrence of nigrostriatal cell death in PD is still controversial.[1,2]

In the present study we measured, as an epiphenomenon of neuronal cell death ex vivo, the Annexin V concentration in cerebrospinal fluid (CSF) of patients with PD and in control subjects. Annexin V belongs to a family of proteins, which in the presence of Ca2+ ions have high affinity to phospholipids.[8] Annexin V binds immediately to phosphatidylserine (PS), a phospholipid which is exposed on dying cells. The rationale for measurement of Annexin V in CSF came from the knowledge that

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**TABLE 1.** *Annexin V concentrations in cerebrospinal fluid of patients with Parkinson's disease, multiple sclerosis, infection, and control subjects*

| Diagnosis | No. | CSF Annexin V (ng/mL) Mean | SD | Significance | p value |
|---|---|---|---|---|---|
| Control subjects | 56 | 2.72 | 1.10 | | |
| MS | 21 | 2.33 | 0.77 | C's vs MS | NS |
| Infection | 10 | 2.88 | 1.42 | C's vs I | NS |
| PD | 96 | 1.99 | 1.22 | C's vs PD : | <0.001 |
| PD+ | 38 | 2.09 | 1.24 | PD+ vs PD : | NS |
| PD− | 58 | 1.92 | 1.22 | PD− vs PD : | NS |

MS, multiple sclerosis; SD, standard deviation; PD, Parkinson's disease; PD+, PD with dementia; PD−, PD without signs of dementia; NS, not significant.

Annexin V adheres to dying cells and may therefore be consumed in situations of massive cell death.[9,10]

## MATERIALS AND METHODS

### Patients

Cerebrospinal fluid samples originated from:

1. Parkinson's disease: 96 patients with a clinical diagnosis of PD. Thirty-eight patients with PD had a Mini Mental State Examination (MMSE) score of <25 at the time of the lumbar puncture as clinical indication of dementia.
2. Multiple sclerosis: 21 patients suspected with demyelinating disease like multiple sclerosis.
3. Infections: 10 patients suspected of having viral or bacterial infections.
4. Control subjects: 56 patients served as control subjects. These patients underwent lumbar puncture because of suspected, but not confirmed, subarachnoid hemorrhage or other indications in the usual neurologic examination, but without signs of dementing or neurodegenerative disease and with normal outcome of routine CSF analysis.

### Measurements of Annexin V

Annexin V concentrations in CSF were measured with a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) according to Jaffe et al.[11] as previously described.[12] In short, this ELISA was devised with a polyclonal IgG antibody (RU-K442) as a capture antibody and a monoclonal antibody (IgG I subclass) as a detection antibody. Recombinant human Annexin V protein was used as standard. The detection limit was 250 pg/mL, intra- and interassay CVs were <10%, and samples were measured in duplicate.

### Statistical Analysis

Mean data are given in nanograms per milliliter with standard deviation between brackets. Statistical analysis was performed with Student's *t* test.

## RESULTS

The Annexin V concentration in the CSF of patients with PD was significantly lower compared with control subjects. As shown in Table 1, the mean value of CSF Annexin V concentration in control subjects was 2.722 (standard deviation 1.099); in patients with PD it was 1.987 ng/mL (standard deviation 1.223 ng/mL). CSF Annexin V concentrations did not correlate with MMSE scores, duration of symptoms of PD, age, sex, or treatment of PD. CSF Annexin V concentrations in patients suspected of having multiple sclerosis (2.33 ± 0.77 ng/mL) and infection (2.88 ± 1.42 ng/mL) did not differ significantly from the concentration found in the control group.

## DISCUSSION

In normal CSF approximately 80% of proteins originate as transudate from plasma and 20% are synthesized by the brain. In case of central nervous system disease, proteins may be derived from damaged nerve cells or produced by inflammatory cells that have entered the central nervous system. All proteins that pass from the blood plasma into CSF do so in inverse relation to their molecular size.[13] Annexin V has a molecular size of 35.000 D, which implies that Annexin V does not pass the intact blood-CSF barrier, but originates from the brain, most likely from the choroid plexus as the main source of CSF.[14] The low concentration of Annexin V in PD may indicate a diminished production or an increased consumption.

Annexin V immediately sticks to dying cells, and it is tempting to suppose that this protein is consumed during neuronal apoptosis, such as has been demonstrated to occur in the midbrain in PD.[3,4] According to our knowledge, the decreased CSF concentrations of Annexin V in PD is the first ex vivo observation that suggests increased neuronal cell death in patients with PD.

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