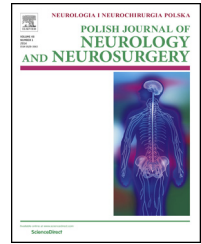


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## Original research article

# The relation between plasma $\alpha$ -synuclein level and clinical symptoms or signs of Parkinson's disease



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## ABSTRACT

**Introduction:** Parkinson disease (PD) is the common neurodegenerative disease.  $\alpha$ -Synuclein (ASN), main aggregating protein in neural cells of CNS in PD, was found in peripheral fluids. Testing ASN in plasma is potential test for diagnose PD, but previous studies are controversial. The aim of this study was to investigate if plasma ASN level may be a valuable biomarker, is the level of plasma ASN concentration different in various motor subtypes of diseases, is there a relation between the level of plasma ASN and the severity of motor symptoms.

**Methods:** Patients with PD hospitalized in Neurology Department, Medical College were performed sequencing the 8th and 9th exon of GBA gene. Next plasma ASN level was tested in 58 patients with sequenced GBA gene and in 38 healthy volunteers (HV), matched by the age (respectively 68.43 vs. 64.57 years of age) and sex (female %, respectively: 43.10 vs.44.74). Patients were assessed with the scales: UPDRS (II, III, IV), Hoehn-Yahr (HY) and qualified to PIGD or TD subtype. For homogeneity of the group patients with GBA mutation were excluded from the analysis.

**Results:** The ASN level did not differ between patients and HV (respectively: 4.53 vs. 3.73 ng/ml) and between patients with different subtypes. There was inverse correlation between ASN and HY in PIGD subtype.

**Conclusions:** Plasma ASN level is not valuable marker of the disease. It does not differ in subtypes of the disease. There is relation between plasma ASN level and the severity of the disease in PIGD subtype.

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## 1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disease characterized by a wide range of clinical features. Because of heterogeneous motor manifestations of early PD some authors suggest three subtypes of the disease: postural instability and gait difficulty (PIGD), tremor dominant (TD) and with mixed symptoms (MPD) [1]. PIGD is associated with worse prognosis, poorer response to treatment, greater risk of dementia and higher prevalence of autonomic disturbances [2]. PD patients differ also in presence and intensity of numerous non-motor symptoms. The most common of them are depression and cognitive impairment. Parkinson's disease dementia (PDD) is the next clinical entity of PD spectrum diseases [3].

The pathogenesis of PD is associated with aggregation of pathologically conformed proteins. The main aggregating protein in PD is  $\alpha$ -synuclein (ASN) [4]. ASN has many physiological functions in central nervous system (CNS). In vivo and in vitro experiments have demonstrated that ASN is released from the cells to the peripheral fluids [5,6]. This was the trigger to search ASN as the biomarker of PD. Finding the objective marker for PD is very important because making an accurate diagnosis of PD is difficult, especially in early stages of the disease. Even 15–25% of patients with parkinsonian symptoms and signs do not have correct diagnosis [7].

The level of ASN in PD patients was investigated in blood [6,8–21] and cerebrospinal fluid (CSF) [22,23], but the results of these studies are controversial. The total plasma ASN in PD patients was found to be higher [9,15], lower [13,16], or similar to the control group [8,11,12,17,24]. Other forms of ASN, such as oligomers [10] or phosphorylated ASN [11–13] were measured also, but the result were also inconclusive. The reasons for that could be associated with different origin of ASN in plasma. Some amount of ASN can originate from erythrocytes and platelets [20]. Min Shi et al. measured the only exosomal ASN, and found that this form of ASN, originated from CNS, is significantly higher in PD patients than in healthy controls [19]. In other study analysing the ratio of ASN oligomers to total erythrocytes' proteins, they explored that it is higher in PD patients than in healthy controls [14].

However there are only a few studies analysing the relation between ASN level in plasma and motor subtypes of PD (PIGD, TD, MPD), cognitive impairment and the presence of other non-motor symptoms [8].

Heterozygous carrier status of glucocerebrosidase (GBA) mutation is thought nowadays to be the most important genetic risk factor for idiopathic PD [25]. The relation between ASN and GBA on the cellular and molecular level has been proven [26]. It is connected with more intensive neurodegenerative process by forming toxic forms of ASN, which influences clinical picture of PD [27]. There are no publications concerning the relation between the plasma ASN concentration and GBA mutation in PD. However the level of plasma ASN oligomers is higher in patients with GD and some other lysosomal diseases than in healthy volunteers. Interesting is the fact that there was no difference of plasma ASN oligomers level between healthy volunteers and patients with GD who were treated with enzyme replacement therapy (ERT) unlike

GD patients without ERT. Moreover the authors mentioned that carriers of GBA mutation with PD had higher level of plasma ASN oligomers than healthy volunteers, but the data were not yet published [18]. In other publication they showed that the relation of dimers/monomers originated from erythrocytes in patients with GD is higher than in healthy volunteers. Dimerisation is restrictive stage of ASN oligomerisation [28]. And finally in the last study they proved reverse correlation between the level of plasma ASN oligomers and the activity of GBA in leukocytes of GD patients [29].

The aim of the study was to evaluate plasma ASN level as a valuable biomarker of PD, and to answer the questions of whether the level of plasma ASN concentration is different in various motor subtypes of diseases (PIGD, TD, MPD) and is there a relation between the level of plasma ASN and if there is a relation between the level of plasma ASN and cognitive impairment or depressive symptoms. Additional aim was to analyze the relation of plasma ASN level between GBA mutation carriers and non-carriers with PD.

## 2. Material and methods

The investigations were carried out on patients diagnosed with PD, hospitalized at the Subdivision of Movement Disorders in Clinical Department of Neurology, University Hospital, etc. in years 2007–2014.

All patients were physically examined by a neurologist and underwent head MR to exclude vascular disease which could cause the Parkinsonism. The actual severity of motor symptoms and the stage of the disease in PD patients group were assessed with UPDRS part II, III and IV as well as Hoehn–Yahr (HY) Scale. Jankovic method was used to classify patient to different motor subtypes of disease (PIGD, TD, MPD) based on UPDRS, part II and III [1]. The Hamilton Depression Rating Scale (HDRS) and Minimal State Examination (MMSE) were used to make the screening for depressive symptoms and cognitive impairment respectively. The blood samples were taken from all of the patients for sequencing the 8th and 9th GBA gene exon with the method preventing amplification of GBA pseudogene, which was the subject of previous publication [30].

Healthy volunteers were invited to the study as the control group (CG). They were selected on the basis of gender and age to match the plasma ASN evaluation group. The subjects of CG group were unrelated members of the patients' families, their minders as well as persons accompanying the patients. They were examined physically by neurologist, than they were assessed with screening tests for cognitive impairment and depressive symptoms. In the CG, there were persons with no neurological disease and severe systemic illness. 5 ml of blood was collected from all participants to test the level of plasma ASN by ELISA. All subjects gave their written informed consent. The study was approved by Bioethics Committee of Medical College, etc. (KBET/106/B/2013, date 28.11.2013).

### Inclusion criteria for PD patients:

- Parkinson's disease diagnosed according to QSBB criteria;
- age above 40 years;

- informed written consent to the examination, obtained individually for stages I and II of the study.

#### Exclusion criteria for PD patients:

- cancer or any other acute or chronic condition demanding proper therapies (antibiotics, steroids, anti-inflammatory drugs, immunosuppressants or immunomodulators, etc.);
- cardio-vascular or respiratory diseases effecting in circulatory or respiratory insufficiency, regardless of the disease intensity;
- hepatic or renal failure or clinical failure of any other organ, diagnosed or suspected in laboratory tests (significantly high transaminase, creatinine, TSH, aggravated anaemia, granulopenia, etc.);
- intensive cerebral ischaemia (basal ganglia and/or white matter) in neuroimaging, suggestive of vascular parkinsonism (in accordance with clinics);
- concomitant CNS diseases (other neurodegenerative conditions, different than PD, motor impairments, SM, epilepsy, etc.);
- concomitant psychiatric disorders demanding treatment with psychotropic drugs;
- history of heavy metal poisoning;
- history of exposure to toxic substances.

#### Inclusion criteria for the control group:

- age above 40 years;
- informed written consent.

#### Exclusion criteria for the control group:

- manifestations of parkinsonism;
- other diagnosed or suspected conditions demanding pharmacological treatment except for well controlled hypertension.

### 2.1. Evaluation of plasma ASN

In order to evaluate plasma ASN concentrations 5 ml circular blood was taken from each of the patients and the healthy controls. Within 15 min the material was centrifuged for 15 min at acceleration  $1500 \times g$  and temperature  $4^\circ\text{C}$ . The plasma collected was stored at  $-80^\circ\text{C}$ . Protein concentration was determined by ELISA immunoenzymatic test, following the product protocol, catalogue no. KHB0061 by Invitrogen. During first incubation, serum ASN bound with solid phase monoclonal antibodies and with the specific polyclonal rabbit antibodies of the liquid phase. Next, after washing out of excessive components, specific polyclonal rabbit antibodies, marked with radish peroxidase, were added where binding with the rabbit antibodies, they complemented a four-layer "sandwich". Following the second incubation and another washing out of the excessive components, a substrata was added to the bound enzyme in order to generate colour. The colour intensity is directly proportional to ASN concentration. ASN levels were given in ng/ml. Each test included two assays where a median value was calculated. If concentration scores obtained from two assays differed substantially, another assay

was performed and the extreme values rejected. The laboratory tests were performed at the Department of Proteomics, ... and financed through a grant by the ... K/ZDS/002288.

### 2.2. Statistical analysis

Calculations were made with the use of IBM SPSS 23.0. software. Evaluation of validity of distribution of the assessed variables with the normal distribution used the Kolmogorov–Smirnov chart and Shapiro–Wilk test. In case a variable showed distribution conforming with normal distribution, comparison made use of t-Student test for two groups and ANOVA for multiple ones. If the variables showed distribution different than the normal one, comparison of groups made use of U Mann–Whitney test (2 groups) or Kruskal–Wallis test (3 or more groups). For evaluation of relation between the quantitative variables, Pearson correlation coefficient was used (for variables valid for normal distribution) or Spearman test (if distribution of variables differed from the normal distribution or for random variables). Chi-square test was used to determine the relation between the ordinal and nominal variable and V-Cramer coefficient to evaluate the effect size. In order to assess the diagnostic usefulness of ASN, the receiver operating characteristic curve (ROC) was drawn. The accepted level of statistical significance was  $p < 0.05$ . In order to preserve the group homogeneity and to eliminate any possible effect of GBA gene mutation, further assays to evaluate relation between plasma ASN and the clinical data, excluded the patients with mutation.

## 3. Results

### 3.1. Demographic data

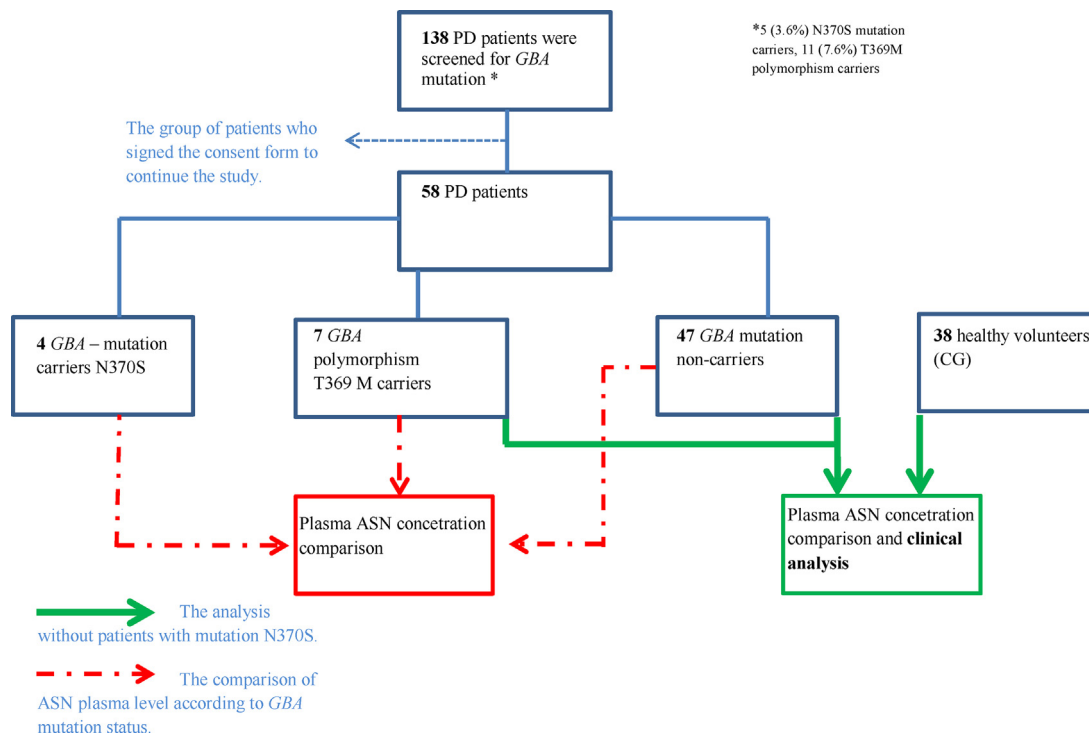
138 patients with PD were screened for GBA mutation. In the whole group of PD patients 16 (11.6%) were carriers of GBA variants. In 5 (3.6%) patients it was N370S mutation and in 11 (7.9%) were T369M polymorphism carriers.

58 PD patients out of screened for GBA mutation group participated in the study of plasma ASN. In this group 4 patients were carriers of N370S mutation and 7 were T369M polymorphism carriers. 1 patient with mutation and 4 with polymorphism did not participated in next stages of the study. In order to preserve the group homogeneity and to eliminate any possible effect of GBA gene mutation, further assays to evaluate relation between plasma ASN and the clinical data were conducted independently for mutation carriers and non-carriers. Finally in clinical analysis participated 54 PD patients without mutation.

Additionally 38 healthy volunteers as CG agreed to participate in the study of plasma ASN. Patients and control group did not differ in the mean age (respectively 68.43 vs. 64.57 years of age) and sex (female %, respectively: 43.10 vs.44.74). Fig. 1 shows the scheme of the study.

### 3.2. Plasma ASN concentrations in the patients and in the healthy volunteers

Based on evaluation of normality of distribution of ASN concentration in the test group and in the controls, it was



**Fig. 1 – The scheme of the study.**

observed that distribution of such concentration differed from the normal distribution and the comparison made use of the nonparametric *U* Mann–Whitney test. The median values of plasma ASN in the patient group and in the test group show no significant difference (respectively: 4.18 vs 3.73 ng/ml;  $p = 0.25$ ).

In order to assess the usefulness of ASN concentrations in PD diagnosis, a ROC curve was drawn. The area under curve (AUC) does not differ statistically from the area below the diagonal (AUC = 0.58;  $p = 0.20$ ), which shows that the variable (ASN concentration) has no predictive value for the diagnosis of PD.

There was no correlation between the age, age of the disease onset or disease duration and plasma ASN in PD patients.

### 3.3. Relation between plasma ASN and intensification of PD symptoms

Intensification of symptoms was measured with the use of UPDRS and HY Scale in 53 out of 58 PD patients, in whom plasma ASN concentration was marked. One patient did not declare the consent for neurological examination. Results of the evaluation making use of subsequent parts of UPDRS Scale (II, III, IV) and HY Scale are presented in Table 1. Validity of distribution of plasma ASN concentration for normal distribution was analyzed in the group of patients with PD at different stage, defined by grades of HY Scale (non-advanced PD: grade 1–2.5; advanced PD: grade 3–5) and in the controls, along with correlation between plasma ASN and intensification of PD manifestations. In the control group plasma ASN showed no normal distribution, therefore comparison of ASN

concentrations among the tested groups made use of the nonparametric Kruskal–Wallis test. No correlation was shown between the average plasma ASN concentration and intensification of motor symptoms measured by subsequent parts of UPDRS. The correlation between plasma ASN and the stage of disease (HY) was on the verge of significance (Spearman correlation  $R = -0.26$ ,  $p = 0.06$ ). Similarly, no difference was shown between the median plasma ASN in patients with different stages of the disease and the healthy controls (Table 2).

### 3.4. Comparison of ASN concentrations in different motor subtypes of Parkinson's disease

Out of 53 patients with PD: 40 were patients with subtype PIGD, 6 with TD subtype and 7 with MPD. The clinical characteristics of patients qualified for individual types are illustrated in Tables 3–5.

Distribution of ASN concentrations in PIGD subtype and in the controls was not valid for normal distribution. Therefore, evaluation made use of nonparametric Kruskal–Wallis test. No significant differences were shown for the median plasma ASN in the investigated subtypes, as compared to the controls ( $p = 0.46$ ).

Correlation between plasma ASN concentration and the clinical data (age, age at onset, duration of the disease, UPDRS II, III, IV, HY) was assessed for each of the tested subtypes (PIGD, TD and MPD). No correlations were found in subtypes TD and MPD. In subtype PIGD, only one average negative correlation of plasma ASN was observed in HY Scale (Spearman's correlation  $R = -0.33$ ;  $p = 0.04$ ).

**Table 1 – Intensification of motor symptoms and advance grade in the tested patient group.**

Variable	Average	SD	Median	Bottom quartile	Upper quartile
<sup>a</sup> UPDRS II	12.90	10.60	10.00	5.00	17.00
<sup>a</sup> UPDRS III	29.51	13.12	27.00	19.00	39.00
<sup>a</sup> UPDRS IV	4.06	3.16	4.00	1.00	5.50
<sup>b</sup> HY	2.40	0.88	2.00	2.00	3.00

<sup>a</sup> Unified Parkinson's Disease Rating Scale.

<sup>b</sup> Hoehn–Yahr Scale.

**Table 2 – The comparison of plasma ASN level in different stages of the disease.**

Group	The level of plasma ASN (ng/ml)				
	Median	Lower quartile	Upper quartile	Mean	SD
Non-advanced PD	4.69	3.05	6.56	4.93	2.35
Advanced PD	3.13	2.20	5.41	3.65	1.70
<sup>a</sup> CG	3.73	2.75	4.86	3.90	1.56

<sup>a</sup> Control group.

**Table 3 – Clinical characteristics of patients with subtype PIGD of the disease.**

Variable	PIGD – 40 patients				
	Average	SD	Median	Minimum	Maximum
Age	69.89	10.36	70.00	49	90
Age at onset	58.56	10.77	58.50	40	83
Duration of disease	10.22	4.78	9.00	3	21
UPDRS II	14.30	11.28	10.00	1	46
UPDRS III	30.41	13.79	27.00	10	62
UPDRS IV	4.29	3.12	4.00	0	11
HY	2.51	0.98	2.50	1	5

**Table 4 – Clinical characteristics of patients with subtype TD of the disease.**

Variable	TD – 6 patients				
	Average	SD	Median	Minimum	Maximum
Age	69.17	9.83	70.00	54	82
Age at onset	61.50	9.57	58.50	50	77
Duration of disease	7.67	3.27	7.00	4	12
UPDRS II	8.83	7.55	7.50	2	22
UPDRS III	29.00	11.17	28.00	16	46
UPDRS IV	3.83	3.76	3.00	0	11
HY	2.25	0.27	2.25	2	2.5

### 3.5. The relation between the level of plasma ASN and cognitive impairment as well as depressive symptoms in PD group and healthy volunteers

The characteristics of PD patients and CG in the context of cognitive impairment and depressive disorder are presented in [Table 6](#). The comparison of plasma ASN level in PD patients with cognitive impairment and without as well as with depressive disorder and without are presented in [Table 7](#). The cut off in MMSE for cognitive impairment was <26 point and in HDRS for depressive disorder was ≥17 point.

There was no correlation (Spearman for non-parametric) between plasma ASN level and HDRS and MMSE score in group of PD patients and CG (see the results in [Table 8](#)).

### 3.6. The relation between the level of plasma ASN and GBA variant status

[Table 8](#) presents the clinical data of PD patients according to GBA status.

There was no difference in plasma ASN concentration between the group of patients with different GBA status ([Table 9](#)).



**Table 5 – Clinical characteristics of patients who did not qualify for the disease subtypes (MPD).**

Variable	MPD – 7 patients				
	Average	SD	Median	Minimum	Maximum
Age	68.86	10.60	67.00	52	85
Age at onset	62.40	12.58	61.00	48	80
Duration of disease	6.00	0.71	6.00	5	7
UPDRS II	10.29	8.75	10.00	1	27
UPDRS III	25.43	12.83	26.00	10	47
UPDRS IV	2.83	3.31	1.50	0	9
HY	1.93	0.61	2.00	1	3

**Table 6 – The screening data of cognitive impairment and depressive symptoms in PD patients and healthy volunteers from control group (CG).**

Mean ± SD	PD (N = 51)	CG (N = 21)	p
MMSE	26.6 ± 4.1	28.5 ± 2.6	0.02
HDRS	7.4 ± 7.3	1.9 ± 3.3	0.02

MMSE – Mnimental State Examination; HDRS – Hamilton Depression Rating Scale.

#### 4. Discussion

In the presented study, the patients with PD showed higher plasma ASN concentration than the healthy controls, however the difference was not statistically significant. The studies published so far have most frequently referred the so-called ASN total, i.e. independent of conformation and the degree of protein aggregation. The presented study evaluated plasma ASN total with the use of commercial ELISA kit, used also for two other assays.

Duran et al. [9] measured the level of plasma ASN total in PD patients, treated and untreated, and in the healthy controls. The groups assessed were slightly larger than those in the present study. The PD patients showed significantly higher ASN level than the controls, regardless of the drugs received. It should be noted however, that the patients and the healthy controls were not selected for age. The controls were younger than PD patients, both, those who were receiving treatment and the untreated ones. The publication fails to quote exact values of the average ASN level in the tested individuals.

Caranci et al. [8] also evaluated plasma ASN total with the use of the same ELISA kit. The tested group and the control one were larger than in the present study and the patients were slightly younger. Included in the study were only patients with the I-III grade of the disease, according to HY Scale. No difference in concentrations was proved between the patients and the healthy controls.

Lee et al. [15] used another commercial ELISA kit to evaluate plasma ASN total and observed statistically higher concentrations in PD patients than in the healthy controls, however the groups were larger than in the present study. Moreover, they showed significantly higher concentration of the protein in 38 patients suffering from the Multiple System Atrophy (MSA) ( $78.1 \pm 3.5$  pg/ml). It was also noted that ASN level was

significantly higher in PD patients, as compared to those with MSA.

As the results of studies in ASN total in PD are contradictory, studies have been initiated to include other ASN forms in plasma and in the cerebrospinal fluid, such as toxic oligomers or phosphorylated ASN (following post-translation modification). The first studies were carried out by El Agnaf et al. [10]. The team created a customized ELISA kit where ASN oligomers were evaluated in 34 PD patients and in 27 controls, selected for age and sex. Nevertheless, the controls were recruited among the patients of the clinical department of haematology and included individuals suffering from cancer, heart diseases, chronic renal failure or cerebral stroke. The PD patients showed statistically higher level of ASN oligomers than the controls, however the authors published no average concentrations.

Many authors emphasize that contradictory results are the effect of contamination of ASN samples originating from morphotic elements of blood (mainly erythrocytes) [20,31]. However, Ishii et al. hypothesized that not only haemolysis contributed to the lack of explicit data, but also the presence of heterophilic antibodies (HA) in human blood. HA have the capacity of reacting with animal immunoglobulins as well as animal borne antigens, which are present in all ELISA kits [14].

Based on the studies quoted, it may be concluded that so far no efficient technique to measure plasma ASN has been designed. Particular studies made use of a wide spectrum of antibodies detecting different ASN forms. The most common was ELISA method. Additionally, Western Blot and Luminex were used. It has not been shown which of the techniques is most useful for ASN detection. Plasma ASN concentrations were found within a very wide range at differentiation from about 80 pg/ml [15] to 0.6 µg/ml [12].

ASN is highly expressed in erythroblasts and reticulocytes. Moreover, haematopoietic transcription factors GATA-1 and GATA-2 induce SNCA expression in erythrocytes. SNCA shows co-expression with four enzymes involved in hem metabolism [32]. About 98–99% blood ASN in healthy individuals originates from erythrocytes. It is not known, however, what is the percentage of distribution of this protein in blood of PD patients [20,31].

The literature quotes remarkably different results of plasma ASN. Obviously, there is a need for an appropriate method to evaluate this protein and to define in which blood sections the differences in concentration are the greatest.

In the literature presented, the patient groups were highly differentiated. It happened that among the recruited controls were individuals with other neurological conditions [10,17]. In

**Table 7 – ASN plasma level (ng/ml) in PD patients with cognitive impairment and with depressive disorder.**

	Cognitive impairment	Depressive disorder	p
Yes	N = 12, median = 3.6, 2.7–5.8 IR	N = 6, median = 6.5, 2.9–8.1 IR	0.7
No	N = 39, median = 4.5, 2.9–6.2 IR	N = 35, median = 4.5, 2.7–6.3 IR	0.3

**Table 8 – The correlation (Spearman) between plasma ASN level and score of cognitive impairment and depressive disorder.**

The relation between plasma ASN level and:	PD (p, rho)	CG (p, rho)
MMSE	p = 0.6, rho = 0.08	p = 0.7, rho = 0.07
HDRS	p = 0.3, rho = 0.16	p = 0.8, rho = 0.08

**Table 9 – Clinical characteristics of PD patients who were N370S mutation carriers (PM), T369M polymorphism (PP) carriers and non-carriers (NC).**

Clinical data	PM (N = 4)	NC (N = 47)	PP (N = 7)	Statistics (p)
Age (years)	68 (65–73.5)	67 (62–75)	73 (61–79)	ns.
Age of onset (years)	56.5 (53–59)	57 (50–66)	64 (56–67)	ns.
Disease duration (years)	12 (10.5–16)	9 (6–12)	9 (6–13)	ns.
UPDRS II (score)	16 (11–25)	10.5 (6–19.5)	5 (3–9)	ns.
UPDRS III (score)	37.5 (28–55.5)	27 (19–37.5)	27 (19–42)	ns.
UPDRS IV (score)	4 (2–7)	4 (1–6)	3 (1–4)	ns.
HY (stage)	2.5 (2–4)	2 (2–3)	2.5 (2–3.5)	ns.
MMSE (score)	23 (20–27)	28 (25–29)	28 (27–30)	ns.

the presented study, the patients and the controls were selected for age and sex. The control group included no individuals with the burden of CNS diseases.

So far scarce contributions have been published to evaluate the relation between ASN in peripheral tissues and the clinical picture of PD. They regarded mainly the motor manifestations. This has been the first study to show a negative correlation between plasma ASN concentration and the grade of the disease (HY) in PIGD subtype. The assays which evaluated such dependency, showed no relation between plasma ASN level and the grade of the disease [8,9,15,21]. It was not investigated, however, if such correlation existed in different subtypes of the disease.

In the study by Shi et al., among the recruited patients, graded 1–4 in HY Scale, the highest values were shown by grade 2 patients with the decreasing tendency along with the more advanced grade. Nevertheless, the correlation was not statistically significant, similarly to the presented study [20]. Caranci also showed no correlation between plasma ASN and the grade of the disease (HY), however enrolled in his study were only grade 1–3 (HY) patients. It was proved that grade 1–2 men showed statistically higher ASN than grade 3 men. Also, negative correlation was observed where the scores were intensified in part IV of MDS-UPDRS for male patients [8].

The relations between ASN concentration and disease stage were also analyzed in research of CSF. Reverse correlation between the stage of disease (HY) and the level of CSF ASN in the group of PD patients was observed [22,23].

Only in one study they analyzed the relation between the plasma ASN level and motor subtype, which were akinetic, with tremor dominance and the one with tremor and bradykinesia. The patients were assessed with MDS-UPDRS Scale, but the method of classification was not specified.

Among 69 patients in 23 they diagnosed akinetic subtype, 30 with tremor dominance and 13 with mixed symptoms. There was no difference of plasma ASN level between subtypes [8].

Kang et al. tested Alzheimer disease (AD) markers (A $\beta$ 1-42, total tau protein: t-tau, phosphorylated tau protein: p-tau) and total ASN in CSF of 63 patients with PD and 39 healthy volunteers. The level of ASN, A $\beta$ 1-42, p-tau was lower in PIGD than in TD subtype. The level of all markers was lower in PIGD subtype, unlike in TD and mixed subtype there was no difference comparing to healthy volunteers. None of the markers revealed any relation to cognitive functioning [22].

In the presented study the patients showed diverse grades of the disease (HY 1–5), therefore it was possible to evaluate correlation with ASN. No differences in ASN concentrations were observed in individuals with lower or higher grade of the disease, yet a decreasing tendency was noted in ASN concentrations along with the growing grade of the disease, however this correlation was on the verge of significance. Subsequent stratification of the analysis revealed significant negative correlation with the grade of the disease (HY) in subtype PIGD. This may suggest that subtype with dominance of axial symptoms should be separated entity.

Despite appropriate selection of the groups in the study performed, no differences in plasma ASN were proved between particular PD subtypes. The study was not devoid of disadvantages. It is likely that the groups designed were too small which prevented showing the significant differences. The size of the sample was restricted mainly by the consent to take blood for plasma ASN evaluation and availability of the valid contact information.

It should be noted however, that the lack of differences in plasma ASN concentrations between the patients and the controls may be due to substantial clinical diversity of the

examined patients. It is confounder for searching the differences. On the other hand it allows to demonstrate the relation between two factors. In presented study following subsequent stratification of the analysis revealed the relation between plasma ASN and disease stage in PIGD subtype.

Similarly to its manifestations, the progression of PD is heterogenous and depends on a variety of factors. The prevalence of particular motor subtypes is not definitely estimated. In the first classification made by Jankovic TD type was up to 55% vs. PIGD 29% [1]. On the other hand last classification based on modified scales MDS-UPDRS showed that the prevalence of TD is up to 24% vs. PIGD up to 64% [33]. The PIGD subtype is often more difficult to treat and with worse reaction to levodopa. Patients in the probe were recruited in specialized towards movement disorders unit and probably this is the reason for large proportion of PIGD subtype in the probe.

The subtype PIGD is associated with more dynamic progression of the disease, as compared to the remaining forms [2]. It may be for this very reason that only this subtype showed correlation between the disease grade and plasma ASN. The negative correlation may be explained by the disease progression, more dynamic at the beginning (1–2.5 HY), yet slowing down at a more advanced stage 3–5 HY [34]. This is coherent with pathological investigations which showed that dopaminergic cells in SNpc decay exponentially with time [35].

Additionally there was no difference of plasma ASN between the group of PD patients with N370S mutation, carriers of T369M polymorphism, non-carriers and healthy volunteers. However there were only 4 PD patients with the mutation and there was observed trend to higher plasma ASN concentration according to the mutation status. Probably the group of mutation carriers was too small. It should be considered if another form of plasma ASN would be better marker for PD connected with GBA mutation, for example oligomers of plasma ASN.

Summing up plasma ASN level is not reliable marker for all types of PD. Further analysis comparing different forms of disease with more participants in all groups and with their stronger homogeneity should be performed. There may be more confounders than the ASN originated from the morphotic elements of blood. It is possible that plasma ASN could be used as the marker of disease progress only in PIGD subtype.

### Conflict of interest

None declared.

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