Parkinsonism and Related Disorders 61 (2019) 203–206

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

Short communication

CSF α-synuclein inversely correlates with non-motor symptoms in a cohort of PD patients

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ARTICLE INFO

Keywords:
α-synuclein
Non-motor symptoms
CSF biomarkers
Parkinson's disease

ABSTRACT

Introduction: Although non-motor symptoms are early and disabling features of PD, reliable predictors and effective therapies are not yet available. Measurement of CSF proteins mirroring brain pathology is currently utilized for diagnostic and prognostic clustering of patients with neurodegenerative diseases but the association with non-motor symptoms in PD has not been evaluated. Here we performed a cross-sectional correlation study, aimed at identifying potential fluid biomarkers for non-motor symptoms in PD.

Methods: CSF levels of 42-amyloid-β, total and phosphorylated tau, α-synuclein and reciprocal ratios were measured in a group of 46 PD patients compared to 37 gender/age-matched controls and correlated with standard clinical scores for motor and non-motor features.

Results: We observed that α-synuclein levels were reduced in PD ($p < 0.05$, AUC = 0.8; $p < 0.05$) and inversely correlated with non-motor symptoms scale total score and items 3 and 9, even independently from age, disease duration, motor impairment severity and dopaminergic treatment ($T = -2,9$, $p < 0.014$; $T = -3.6$, $p < 0.05$; item 9: $T = -2.1$, $p < 0.05$, respectively).

Conclusions: Our findings suggest that the reduction of CSF α-synuclein may parallel degeneration of non-dopaminergic systems. Although confirmatory studies are necessary, CSF α-synuclein reduction might represent a potential biomarker to monitor non-motor symptoms burden.

1. Introduction

In Parkinson's diseases (PD), the cardinal motor signs (MS), tremor, rigidity and bradykinesia, are essentially related to the loss of dopaminergic neurons in *Substantia Nigra pars compacta* (SNpc), whereas the heterogeneous spectrum of non-motor symptoms (NMS) appears to be determined by diffuse neurodegenerative processes, involving multiple neurotransmitter systems [1].

While treatments and biomarkers are currently available for MS, NMS, although responsible for poor quality of life and severe disability in patients, still lack effective therapies and reliable predictors [1]. Recently, different clinical and biochemical variables have been associated to NMS progression [2]. However, to date, the available data are still preliminary, thus highlighting the urgent need to identify potential biomarkers, in order to detect and monitor the evolution of NMS in PD.

Quantitative analysis of neurodegeneration-related proteins within CSF allows tracking pathological changes of the brain, supporting the early differential diagnosis and, in some cases, the prognostic clustering of patients with neurodegenerative diseases [3,4]. Indeed, the comprehension of pathogenic mechanisms underlying clinical features, together with the identification of related biomarkers, are of fundamental importance to identify successful, personalized therapeutic approaches.

In this cross-sectional study, we hypothesized that widespread degeneration underlying NMS might mirror in CSF proteomics. Therefore, we evaluated, in a cohort of PD patients, the association between NMS severity and a panel of CSF proteins, including α-synuclein (a-syn), 42-amyloid-β peptide ($A\beta_{42}$), total and 181-phosphorylated tau (t-tau and p-tau) and reciprocal ratios, aimed at exploring their potential value as NMS biomarkers.

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https://doi.org/10.1016/j.parkreldis.2018.10.018
Received 10 April 2018; Received in revised form 2 October 2018; Accepted 15 October 2018

2. Methods

2.1. Subjects

The study was performed at Tor Vergata University Hospital (Rome, Italy) and involved a total of 83 subjects, grouped in PD (n = 46) and control (CTL, n = 37). PD patients were diagnosed by using UK-PDSBB diagnostic criteria and prospectively collected from 2015 to 2017 (DaT-SPECT further supported clinical diagnosis in > 50% of patients). CTL group included age/sex-matched subjects, with other non-neurodegenerative conditions (suspect of vascular or inflammatory diseases, neuropathies or radiculopathies) without signs of motor and cognitive impairment, receiving lumbar puncture (LP) for diagnostic purpose. In agreement with previous studies [3], in order to improve diagnostic accuracy, subjects showing values suggestive of Alzheimer's Disease (AD) were excluded from the study (AD: $A\beta_{42}$/p-tau < 6.4). For all included subjects, demographic data and medical history were recorded. At the same time of CSF sampling, a movement disorder specialist assessed PD patients with the standard clinical scores for MS (Unified Parkinson Disease Rating Scale part 2 and 3, UPDRS2-3), NMS (Non Motor Symptoms Scale, NMSS, total and single item scores) and cognition (Mini Mental State Examination MMSE, adjusted for age and educational level); the personal levodopa equivalent daily dose (LEDD) was finally calculated for each PD patient. Assessment was conducted under the effects of habitual antiparkinsonian medication. LP and CSF analysis were performed according with standard procedures, as previously described [3]. Samples with > 4 cells or blood contamination were preliminarily excluded from the study to avoid bias. All procedures were conducted in agreement with the "good clinical practice" indications and to either institutional/local ethical standards or ethical principles of Helsinki declaration. Informed consent was obtained from each participant included in the study.

2.2. Biomarkers assay

Levels of CSF biomarker t-tau, p-tau and $A\beta_{42}$ were assessed by utilizing commercially available sandwich enzyme-linked immunosorbent assays (ELISA), following standard procedures (Innotest, Fujirebio). Level of a-syn was determined by using alpha-synuclein ELISA kit (Covance). Benchmark Plus (Biorad) and Synergy HT (Biotek) plate readers were used for analysis. $A\beta_{42}$/p-tau, p-tau/t-tau, t-tau/a-syn, p-tau/a-syn, t-tau/a-syn + $A\beta_{42}$, p-tau/a-syn + $A\beta_{42}$ ratios were also calculated as described [5].

2.3. Statistical analysis

The distribution of collected variables was evaluated with the Shapiro-Wilk test. Group differences in demographics and clinical parameters were tested with parametric (one-way ANOVA) or non-parametric tests (chi-square test, U-Mann-Whitney test), as appropriate. Differences in CSF biomarkers levels were measured with one-way MANOVA test, adjusting the model with the main covariates age and gender. Differences in biomarkers ratios were finally tested with one-way ANOVA analysis. ROC (receiver operating curve) analysis and cut-off points calculation were performed to estimate diagnostic sensitivity (Se) and specificity (Sp) values of biomarkers. Correlations between demographic/clinical features and biomarkers were explored with Spearman's test and successively confirmed through linear regression analysis, also by using age, disease duration, motor impairment severity (UPDRS 3 score) and LEDD as covariates. Power analysis for sample size calculation was preliminary conducted by using published data. Statistical significance was set at $p < 0.05$. Statistical analysis was performed by using IBM-SPSS-22.

Table 1
Study population data. n = number, M = male, F = female. Age and disease duration is expressed in years; $A\beta_{42}$, t-tau, p-tau, a-syn concentrations are in pg/ml.

	PD		CTL		significance
n	46		37		
gender M/F	24/22		22/15		no
	mean	*SD*	*mean*	*SD*	
age	57.4	9	60.9	10.1	no
$A\beta_{42}$	779.4	242.5	862.9	224.9	no
t-tau	140.5	51.3	257.9	52.3	*p < 0.0001*
p-tau	35.5	11.3	40.1	7.2	no
a-syn	927.2	378.5	1376.9	358.5	*p < 0.05*
Aβ42/p-tau	22.8	6.9	21.4	4.7	no
p-tau/t-tau	0.22	0.1	0.2	0.04	*p < 0.01*
t-tau/a-syn	0.17	0.06	0.2	0.1	no
p-tau/a-syn	0.04	0.01	0.03	0.02	no
t-tau/a-syn + $A\beta_{42}$	0.09	0.03	0.14	0.03	*p < 0.01*
p-tau/a-syn + $A\beta_{42}$	0.02	0.004	0.02	0.005	no
disease duration	1.2	0.8	–	–	–
UPDRS 2	5.3	2.1	–	–	–
UPDRS 3	13	3.6	–	–	–
MMSE	29.3	1.2	–	–	–
NMSS	24	9.5	–	–	–

3. Results

Demographic and clinical data of the study population are summarized in Table 1. The groups were homogeneous in age and gender distribution. One-way MANOVA demonstrated significant difference in CSF biomarkers between the groups [$F(4,21) = 12.8$, $p < 0.0001$; Wilk's $\Lambda = 0.22$, partial $\eta^2 = 0.78$). PD exhibited both lower a-syn (927.2 ± 378.5 pg/ml vs 1376.9 ± 358.5; $p < 0.05$) and t-tau levels (140.5 ± 51.3 pg/ml vs 257.9 ± 52.3; $p < 0.0001$) compared to the CTL group, independently from age and gender. One-way ANOVA analysis revealed that p-tau/t-tau ratio was significantly higher in PD than CTL (0.22 ± 0.08 vs 0.17 ± 0.05; $p < 0.01$), whereas t-tau/a-syn + $A\beta_{42}$ ratio was lower (0.09 ± 0.03 vs0.14 ± 0.03; $p < 0.01$). Diagnostic accuracy was determined by the ROC curve analysis for significantly different biomarkers. a-syn (AUC = 0.8; $p < 0.05$, 95% CI 0.6–0.99), at the cut-off value of 1143 pg/ml showed 86% sensitivity (Se) and 77% specificity (Sp). t-tau (AUC = 0.7; $p < 0.005$, 95% CI 0.6–0.8), at the cut-off value of 190 pg/ml showed 80% Se and 65% Sp. p-tau/t-tau ratio (AUC = 0.7; $p < 0.005$, 95% CI 0.6–0.8), at the cut-off value of 0.185 showed 70% Se and 65% Sp. t-tau/a-syn + $A\beta_{42}$ ratio (AUC = 0.8; $p < 0.005$, 95% CI 0.67–1), at the cut-off value of 0.1005 showed 88% Se and 60% Sp. Correlation analysis revealed that a-syn inversely correlates with either NMSS total score ($R = -0.56$, $p < 0.05$) or single item score number 3 "mood/cognition" ($R = -0.57$, $p < 0.05$) and number 9 "pain/smell/weight/sweating" ($R = -0.51$, $p < 0.05$). The linear regression analysis further confirmed such association, even independently from age, disease duration, motor impairment severity and total LEDD (NMSS total score: $T = -2.9$, $p = 0.014$; item 3: $T = -3.6$, $p < 0.005$; item 9: $T = -2.1$, $p < 0.05$). Also p-tau inversely correlated with NMSS total score ($R = -0.5$, $p < 0.05$; $T = -2.9$, $p < 0.01$) and item 3 score ($R = -0.5$, $p < 0.05$; $T = -1.9$, $p = 0.07$, not significant). Finally, a-syn and t-tau showed a direct correlation ($R = 0.44$, $p < 0.05$; $T = 4.1$, $p < 0.001$). No further significant relationships emerged between clinical parameters (UPDRS 2–3, MMSE, disease duration) and single biomarker levels or ratio values.

4. Discussion

This preliminary study demonstrates an inverse association between CSF total a-syn levels and NMS in PD patients, independent from age,

disease duration, motor impairment severity and dopaminergic treatment. Consistent with current literature [4–6], we found a reduction of CSF total a-syn which can be referred to the protein accumulation within Lewy bodies [4]. Indeed, in PD, Lewy pathology may selectively affect the cortex, the olfactory bulb, the dorsal motor nucleus of vagus, the basal forebrain and the brainstem nuclei, depending on complex and largely unknown mechanisms of neuronal vulnerability [7]. The decrease of CSF-circulating a-syn could thus underlie widespread synucleinopathy in a number of brain regions that represent the anatomical substrate of NMS [1,7]. In particular, we found significant correlations between a-syn and either item 3 or 9 of NMSS, assessing mood/cognition and pain/smell/weight/sweating respectively, which in turn indicate a prominent impairment of neural networks controlling these functions. Although multiple transmitter systems are involved, the pathophysiology of such disturbances might implicate the selective loss of the cholinergic innervations in the mesocorticolimbic pathway [1]. Of interest, the dysfunction of cortical cholinergic activity in PD can occur even in the absence of neuronal loss, as a consequence of the accumulation of a-syn into the basal forebrain cholinergic neurons [8]. Indeed, abnormal forms of a-syn are able to alter cholinergic signaling since the early phases of the disease, irrespective of the dopaminergic deficit [9]. Our data overlap this preclinical evidence, and collectively suggest that, in PD, the reduction of CSF a-syn might parallel its deposition within neuronal networks involved in non-motor functions. Additionally, recent results from Kang and colleagues show that lower CSF a-syn levels correlate with poorer cognitive test performances in *de novo* patients [10], whereas Goldman and coworkers demonstrated the association between low a-syn and gait/postural complications in advanced PD patients [6]. Moreover, a study of functional neuroimaging showed how CSF a-syn level correlates whit cortical dysfunction beyond the nigrostriatal dopaminergic denervation, reflecting the impairment in non-motor cortical functional connectivity networks [11]. Altogether, these lines of evidence strengthen the hypothesis of an inverse correlation between a-syn and NMS severity. Consistently, we did not find significant relationships between a-syn and MS rated by UPDRS 2–3 scores. Moreover, the statistical model was adjusted for the main determinants of NMS burden (LEDD, age, motor impairment severity and disease duration), further highlighting the independent association of a-syn and NMSS.

It is interesting to note that similar independent associations were obtained for p-tau. Indeed CSF levels of p-tau, although within a normal range, directly correlated with a-syn and inversely correlated with NMSS total score and item 3 score, probably because of the synergistic molecular interactions of the two proteins, leading to the co-aggregation into the Lewy bodies [12]. Finally, it should be remarked the relevance of the increased p-tau/t-tau ratio as a peculiar CSF trait of PD. Similar values have been indeed recently measured in a larger population, though unrelated to clinical severity [2].

A note of caution is required in the interpretation of these data and the apparent discrepancy with two other recent interesting studies [2,6]. The small sample size, the adopted outcome measures, the inclusion criteria and the disease duration of enrolled subjects, could explain, at least in part, the different results in both “PPMI” and “BioFIND” cohorts. Of note, these studies involved either newly diagnosed drug-naïve patients [2] or older, moderate-advanced, treated patients [6], respectively, whose CSF a-syn levels were not associated with NMS rated with the MDS-UPDRS Part I score or RBD questionnaire total score. The different clinical-demographic features of the populations indeed might account for a different CSF proteomic profile; likewise, also the use of the specific NMS rating scale (NMSS) should be considered in the comparison of current results with the existing literature.

Additionally, it should be reminded that our control group did not include healthy volunteers, but patients receiving LP for diagnostic purpose, in the absence of neurodegenerative diseases.

Our findings are certainly preliminary and highlight the limitations of the spot measurement of biomarkers. Larger longitudinal studies, possibly integrated with neurophysiological and/or imaging investigations are thus mandatory to confirm these initial observations. Nevertheless, our data, in line with previous studies [6,10], suggest that CSF a-syn levels may track some NM features of PD.

5. Conclusions

NMS still lack reliable biomarkers for monitoring and predicting disease progression. This study demonstrates an inverse and independent association between NMS and total a-syn CSF levels, in the absence of significant correlations with MS. We suggest that the decrease of CSF a-syn levels mirrors a widespread degenerative process involving non-dopaminergic networks. In this perspective, measurement of total CSF a-syn may represent a biomarker for NMS, supporting the assessment of frailty in PD patients. Definitely, larger confirmatory studies are required.

Declarations of interest

None.

Financial disclosure

The authors declare no conflict of interests. The study was partially funded by the Italian Ministry of Health GR-2011-02349822.

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