The Canadian Journal of Neurological Sciences (2024), **51**, 573–576
doi:10.1017/cjn.2023.281

Canadian Journal of Neurological Sciences
Journal Canadien des Sciences Neurologiques

Brief Communication

Disease Progression and Sphingolipids and Neurofilament Light Chain in Early Idiopathic Parkinson's Disease

Blas Couto[1,2], Mario Sousa[3,4], Paulina Gonzalez-Latapi[5], Eric McArthur[6], Anthony Lang[1], Alice Chen-Plotkin[7] and Connie Marras[1]

[1]Edmond J. Safra Program in Parkinson's Disease, Rossy Program for PSP Research and the Morton and Gloria Shulman Movement Disorders Clinic, Toronto Western Hospital, Toronto, ON, Canada, [2]Institute of Cognitive and Traslational Neuroscience (INCyT), at the INECO-CONICET-Favaloro University Hospital, Buenos Aires, Argentina, [3]Department of Neurology, Inselspital, Bern University Hospital, Bern, Switzerland, [4]Graduate School for Health Sciences, University of Bern, Bern, Switzerland, [5]Ken and Ruth Davee Department of Neurology, Northwestern University, Feinberg School of Medicine, Chicago, IL, USA, [6]London Health Sciences Centre, London, ON, Canada and [7]Department of Neurology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

ABSTRACT: Parkinson's disease(PD) lacks a biomarker for disease progression. To analyze how cerebrospinal fluid (CSF), glucosylceramide (GlcCer), sphingomyelin (SM), or serum neurofilament light chain (NfL) associate with progression of PD in a retrospective cohort, we used linear mixed-model regressions between baseline biomarkers and change in dopamine transporter brain-imaging (DaTscan©), Montreal cognitive assesment (MoCA), or global composite outcome (GCO) score. In 191 PD patients, biomarkers were not associated with DaTscan or MoCA change over 2.1 years. Higher baseline GlcCer/SM ratio and serum-NfL nonsignificantly associated with increase in GCO score. Results do not support a role for CSF-sphingolipid/serum-NfL to predict cognitive and DaTscan progression in early-PD. Potential prediction of global clinical change warrants further study.

RÉSUMÉ : Il n'existe pas de biomarqueur de la progression de la maladie de Parkinson (MP). Pour analyser comment le glucosylcéramide (GlcCer) et la sphingomyéline (SM) du liquide cérébrospinal (LCS) ou encore les neurofilaments à chaîne légère (NFCL) du sérum sont associés à la progression de la MP dans une cohorte rétrospective, nous avons utilisé des régressions linéaires à modèle mixte entre les biomarqueurs de base et l'évolution de résultats obtenus lors d'examens d'IRM du transporteur de la dopamine (DaTscan©) ainsi qu'au moyen du *Montreal Cognitive Assessment* (MoCA) ou du score *Global Composite Outcome* (GCO). Chez 191 patients atteints de la MP, les biomarqueurs n'ont pas été associés à l'évolution des résultats du DaTscan© ou du MoCA au cours d'une période de 2,1 ans. Un rapport GlcCer/SM de base plus élevé et les NFCL du sérum ont par ailleurs été associés de manière non notable à l'augmentation du score GCO. Ces résultats ne confirment donc pas le rôle de la SM du LCS et des NFCL dans la prédiction de la progression cognitive et des résultats au DaTscan© au début de la MP. En somme, la prédiction potentielle de l'évolution clinique globale de cette maladie mérite d'être étudiée plus avant.

Keywords: Parkinson disease; progression; spect; cognition; sphingolipid; biomarkers

(Received 21 April 2023; final revisions submitted 20 August 2023; date of acceptance 21 August 2023; First Published online 29 August 2023)

A current unmet need in Parkinson's disease (PD) is a biological marker that predicts disease progression from a baseline measurement more accurately than the currently used pure clinical assessment.[1] A link between PD and sphingolipid metabolism and lysosomal pathways has been highlighted by numerous clinical and basic science observations,[2,3] including glucocerebrosidase (*GBA*) acting as a genetic risk factor for developing PD[4] along with other lysosomal genes (*SMPD1*),[5] and reports of altered levels of glucosylceramide (GlcCer) and sphingomyelin (SM) in the *substantia nigra* (SN) and plasma of patients with PD.[6,7] Levels of these sphingolipids in plasma and cerebrospinal fluid (CSF) have been correlated with progression of motor and cognitive features of PD.[8] If replicated in other studies, GlcCer or SM levels could potentially be used to identify patients at risk of more aggressive disease progression. Additionally, these shared pathways could be targeted by therapies influencing sphingolipid production or accumulation.

Similarly, although not specific for PD, neurofilament light-chain (NfL) in CSF and in serum is one of the most promising biomarkers of disease severity and progression and has been suggested as a potential marker to assess response to disease-modifying treatments.[9]

Previous studies measuring sphingolipids in PD analyzed disease progression mainly based on clinical outcomes, which is influenced by medication and non-PD-related factors.[8,10] In contrast, dopaminergic denervation of the striatum, measured

Corresponding author: C. Marras; E-mail: connie.marras@uhnresearch.ca

Cite this article: Couto B, Sousa M, Gonzalez-Latapi P, McArthur E, Lang A, Chen-Plotkin A, and Marras C. (2024) Disease Progression and Sphingolipids and Neurofilament Light Chain in Early Idiopathic Parkinson's Disease. *The Canadian Journal of Neurological Sciences* **51**: 573–576, https://doi.org/10.1017/cjn.2023.281

using dopamine transporter (DAT) 123-I Ioflupane (DaTscan©) single-photon emission computed tomography imaging, is a biological readout and potentially more sensitive marker of disease progression in early-stage patients.[11]

Here, we examined whether CSF sphingolipids and serum NfL at baseline were associated with the change over time in putaminal dopaminergic denervation in patients with early-stage PD or with their clinical progression assessed with the global composite outcome (GCO), a multidomain clinical measure of disease severity. As secondary outcomes, we evaluated rate of caudate dopaminergic denervation since it has been recently demonstrated that early caudate dopaminergic denervation is associated with faster cognitive decline in this PPMI cohort,[12] and global cognitive performance measured by the Montreal Cognitive Assessment (MoCA).

Participants. Newly diagnosed, unmedicated patients with PD were included from the Parkinson's Progression Markers Initiative (PPMI) cohort. The PPMI is an ongoing international, multicenter observational cohort study aimed at identifying serological, genetic, spinal fluid, and imaging biomarkers of PD progression. Participants with CSF biomarkers and longitudinal DaTscan© imaging data were included (see supplementary flow diagram 1).

Clinical data. Clinical data were extracted from all available visits. Nonmotor and motor symptoms and motor signs were measured using the Movement Disorders Society endorsed-Unified Parkinson's Disease Rating Scale (MDS-UPDRS-parts I-III); activities of daily living using the Schwab and England (ADL); global cognition using the MoCA. Clinical progression of disease was assessed by change in the GCO. The GCO weights nonmotor symptoms, motor symptoms, motor signs, overall activities of daily living, and global cognition, standardized by averaging the z-scores of each component. As previously described,[13] we calculated GCO change using the mean/SD from the baseline scores as reference.

Biomarkers. GlcCer and SM were quantified at baseline from CSF samples by liquid chromatography-tandem mass spectrometry (LC–MS/MS) in human CSF (SANOFI, PPMI Project ID: 135). NfL at baseline was measured in serum samples from the PPMI sample collection on the Simoa singleplex NF light assay (University Medical Center Goettingen and Paracelsus Elena-Klinik, Kassel, Germany; PPMI Project ID: 144) (see Supplementary material for detailed storage time).

Presynaptic dopamine transporter (DAT) scan. Image acquisition and post-processing are described in the imaging technical operations manual. Putamen and caudate specific binding ratio (SBR) ipsilateral to the more affected side of the body based on the limb-related items of the MDS-UPDRS-part III were used instead of the contralateral side based on a higher sensitivity to detect change on the least affected (ipsilateral) compared to contralateral putamen.[11] DatScan data were collected from available analyzed LONI repository of PPMI, and we used baseline/screening visits and longitudinal follow-up visits at 12, 24, and 48 months (visit http://ppmi-info.org for the technical manual, file PPMI_DatScan_SPECT_Image_Processing_SBR_Calculation_Methods_20130823).

Statistical analysis. The associations between CSF sphingolipids and serum NfL at baseline and the primary and secondary outcomes were estimated using linear mixed models with covariates of time (per year), the log-transformed biomarker, and an interaction term between time and the log-transformed biomarker, adjusted for age, sex, and baseline GCO score. Due to non-normality of residuals in the DaTscan values, they were log-transformed. A two-sided p-value<0.05 was considered significant. All analyses were completed using R version 4.1.1.

Table 1: Demographic and clinical information, sphingolipid and NfL measurements at baseline

	Baseline ($n = 191$)
Age (years)	62 (9.4)
Gender female *n* (%)	67 (35)
Disease duration in months	6.3 (6.1)
MDS-UPDRS-part III score	23 (8.7)
MoCA score	27 (2.4)
CSF GlcCer	6.1 (5.3)
CSF SM	610 (240)
CSF GlcCer/SM ratio	0.010 (0.0059)
Serum NfL	14 (9.8)

CSF = cerebrospinal fluid; GlcCer = glucosyl ceramide; MDS-UPDRS = movement disorders society endorsed unified Parkinson disease rating scale; MoCA = Montreal cognitive assessment; NfL = neurofilament light chain; SM = sphingomyelin.

Standard protocol approvals, registrations, and patient consents. The study was approved by the institutional review board at each PPMI site. Written informed consent for research was obtained from all participants in the study.

A total of 191 patients with a median follow-up of 2.1 years were analyzed (see table 1 for additional features). Primary outcomes: Unadjusted SBR in the ipsilateral putamen declined by a mean of 7.25% per year. After adjusting for age, gender, and baseline GCO score, no relationships were found between change in ipsilateral putamen SBR and baseline CSF GlcCer (time*log(CSF GlcCer) estimate = 1.00; [95%-CI:0.98, 1.01]; $p = 0.62$), CSF SM (time*log(CSF SM) estimate = 0.99; [95%-CI:0.98, 1.01]; $p = 0.27$), GlcCer/SM ratio (time*log(GlcCer/SM ratio) estimate = 1.00; [95%-CI:0.99, 1.02]; p=0.50), and serum NfL (time*log(NfL) estimate = 0.99; [95%-CI:0.97, 1.01]; $p = 0.26$; supplementary table 1). After adjusting for age, sex, and baseline GCO-score, a higher ratio of CSF GlcCer/SM (time*log(GlcCer/SM ratio) estimate = 1.01; [95%-CI:1.00, 1.02]; p=0.07) and higher serum NfL (time*log(NfL) estimate = 1.02; [95%-CI:1.00, 1.04]; $p = 0.08$) associated with a greater increase in the GCO-score per year. Secondary outcomes: We found no association between CSF sphingolipids or serum NfL and either MoCA score or ipsilateral caudate SBR (supplementary table 2).

The main results of this study are the lack of association of the baseline CSF-sphingolipid biomarkers or serum NfL with dopaminergic imaging markers of disease progression in patients with early-stage iPD over a median follow-up of 2.1 years. However, there was a suggestion (nonstatistically significant) that a higher level of serum NfL ($p = 0.08$) and higher CSF GlcCer/SM ratio ($p = 0.07$) would associate with greater clinical progression (GCO score) over time. Therefore, it is possible that either CSF GlcCer/SM ratio or serum NfL could predict the change in GCO score with a larger number of subjects and more homogeneous cohort with respect to symptomatic treatment. This is supported by a relatively higher magnitude of change in GCO score during the first year of follow-up (2.7%), where most of the patients were still untreated for symptoms, compared to smaller changes by the 2nd and 4th year (Fig. 1), where a great proportion of the patients were under dopaminergic replacement treatment. The change in

https://doi.org/10.1017/cjn.2023.281

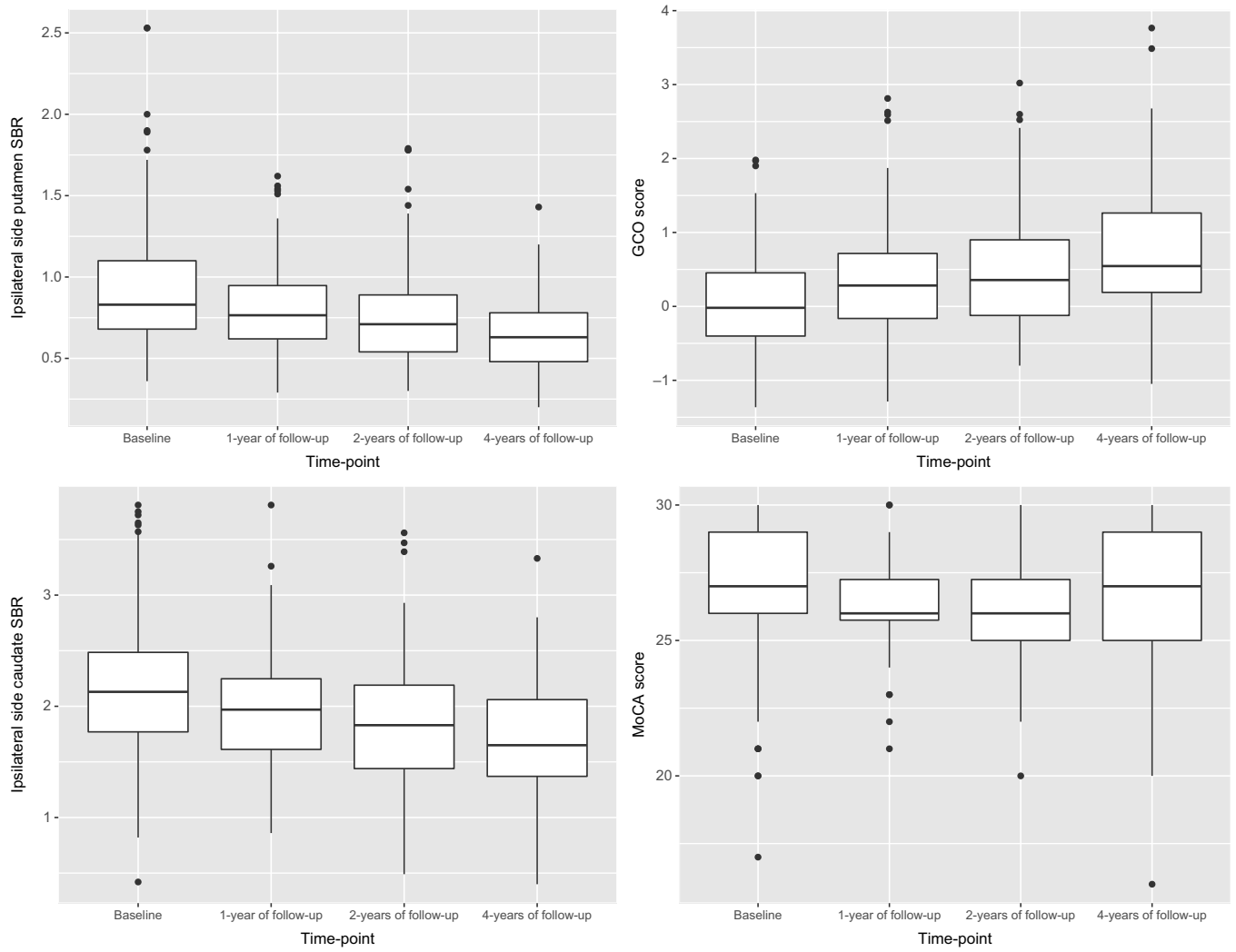

Figure 1: Longitudinal data represented in boxplots for DaTscan, GCO score, and MoCA.

the GCO score in the first year represent a true change in underlying disease compared with the subsequent time points. Additionally, inclusion of longitudinal measurements of these biomarkers might be needed to demonstrate significant association with the magnitude of change of GCO.

In secondary analyses, the changes in MoCA and ipsilateral caudate SBR over time were not associated with CSF sphingolipid biomarkers or serum NfL. Our results do not replicate the associations found in a previous analysis of the same cohort[8] that found that patients in the top quartile of GlcCer/SM ratio at baseline showed a faster decline in MoCA during longitudinal follow-up compared with those in the lowest quartile. The authors did not adjust for disease severity at baseline which may account for the difference, if CSF GlcCer/SM ratio at baseline is associated with more aggressive initial manifestations of the disease. In our analyses, neither sphingolipids nor NfL predicted the change in ipsilateral caudate SBR.

We found that both serum NfL and CSF GlcSer/SM ratio showed association with GCO change over time, but this did not reach statistical significance. In keeping with this, a single-center study that followed 118 patients with PD with longitudinal UPDRS-III showed that higher plasma NfL at baseline predicted faster increase in motor scores and faster decrease in memory scores, but MoCA was not used for these analyses. Additional findings of another study[14] generated from 376 patients of the same cohort showed an association between higher baseline NfL levels and greater changes in motor scores and DaT imaging over time, but no association with MoCA scores. Although patient selection was different to our study, these discrepancies highlight the substantial variability of results within the same cohort, particularly when combining different subsets of data and contrasting them against different outcomes. It suggests that there is significant interindividual variability in the relationships between the biomarkers studied and motor and cognitive outcomes, possibly reflecting heterogeneity of the underlying biology of PD. Better understanding the determinants of the relationships will be critical for patient stratification for research and clinical trials. Of note, our results also point to the usefulness of the GCO score as an outcome of early-PD progression and patient stratification as previously demonstrated.[15]

Our results have implications for the potential use of these biomarkers as outcomes of disease progression in clinical trials aiming to enroll patients with early PD. As new pharmacological targets for disease modification pathways are defined and tested in patients with clinical diagnosis confirmed by DaTscan, a careful appraisal is needed on evidence related to predicting which

https://doi.org/10.1017/cjn.2023.281

patients have a higher biological vulnerability and are prone to a faster decline. Our results stress the need for replication and consistency of evidence across cohorts and analyses within cohorts. Furthermore, it is also possible that PD is sufficiently biologically heterogeneous that CSF sphingolipid biomarkers might be more useful to predict progression in selected subsets of patients that would need to be defined to allow proper enrichment of the study population for a future clinical trial of a potential disease modifying therapy targeting these cellular pathways.

Strengths of our analyses include accounting for baseline disease severity, the choice of a biological outcome (DaTscan) that is less influenced by the effect of dopaminergic replacement therapies and that has been shown to correlate well with part III and total scores of MDS-UPDRS in early stages of the disease,[12] as well as the inclusion of a newer measure of global (motor + nonmotor) disease progression.

Our results do not support an association between baseline CSF sphingolipid measures or serum NfL and striatal DaTscan© SBR, or MoCA scores. However, a potential trend was observed between both CSF GlcCer/SM ratio and serum NfL at baseline and GCO score change over time. The GCO may be a more sensitive marker of clinical progression than MoCA or striatal DaTscan changes alone, particularly in early PD, combining measures of motor, cognitive, and autonomic symptoms. These results support incorporating composite clinical outcomes such as GCO-score in future cohorts.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/cjn.2023.281.

Acknowledgments. Data used in the preparation of this article were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/access-data-specimens/download-data). For up-to-date information on the study, visit ppmi-info.org. PPMI – a public–private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including including AbbVie, Allergan, Amathus Therapeutics, Avid Radiopharmaceuticals, Biogen, BioLegend, Bristol Myers Squibb, Celgene, Denali Therapeutics, GE Healthcare, Genentech, GlaxoSmithKline plc., Golub Capital, Handl Therapeutics, Insitro, Janssen Neuroscience, Eli Lilly and Company, Lundbeck, Merck Sharp & Dohme Corp., Meso Scale Discovery, Neurocrine Biosciences, Pfizer Inc., Piramal Group, Prevail Therapeutics, Roche, Sanofi Genzyme, Servier Laboratories, Takeda Pharmaceutical Company Limited, Teva Pharmaceutical Industries Ltd., UCB, Verily Life Sciences, and Voyager therapeutics Inc.

Funding. Dr Marras is a site PI for the PPMI study and receives funding from the Michael J Fox Foundation for this role.

Competing interests. None.

Statement of authorship. BC: Conceptualization, data curation, investigation, writing – original draft (Lead), writing – review and editing.

MS: Conceptualization, data curation, Investigation, writing – original draft (Lead), writing – PGL: conceptualization, methodology, investigation, writing – review and editing.
EM: Methodology, data curation, writing – review and editing.
ACP: Conceptualization, writing – review and editing.
AEL: Conceptualization, supervision, writing – review and editing.
CM: Conceptualization, supervision, writing – review and editing.

References

1. Espay A, Kalia L, Gan-Or Z, et al. Disease modification and biomarker development in Parkinson disease: revision or reconstruction? Neurology. 2020;94:481–94. DOI: 10.1212/WNL.0000000000009107.
2. Robak L, Jansen I, van Rooij J, Uitterlinden A, Kraaij R, Jankovic J. Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. Brain. 2017;140:3191–203. DOI: 10.1093/brain/awx285.
3. Nguyen YC, Wong D, Ysselstein A, et al. Mitochondrial, and lysosomal dysfunction in Parkinson's disease. Trends Neurosci. 2019; 42:140–9. DOI: 10.1016/j.tins.2018.11.001.
4. Chahine L, Qiang J, Ashbridge E, et al. Clinical and biochemical differences in patients having Parkinson disease with vs without GBA mutations. JAMA Neurol. 2013;70:852–8. DOI: 10.1001/jamaneurol.2013.1274.
5. Alcalay R, Mallett V, Vanderperre B, et al. SMPD1 mutations, activity, and α-synuclein accumulation in Parkinson's disease. Mov Disord. 2019;34:526–35. DOI: 10.1002/mds.27642.
6. Huebecker M, Moloney E, van der Spoel A, et al. Reduced sphingolipid hydrolase activities, substrate accumulation and ganglioside decline in Parkinson's disease. Mol Neurodegener. 2019;14:40. DOI: 10.1186/s13024-019-0339-z.
7. Galper J, Dean N, Pickford R, et al. Lipid pathway dysfunction is prevalent in patients with Parkinson's disease. Brain. 2022;145:3472–87. DOI: 10.1093/brain/awac176.
8. Huh Y, Park H, Chiang M, et al. Glucosylceramide in cerebrospinal fluid of patients with GBA-associated and idiopathic Parkinson's disease enrolled in PPMI. NPJ Parkinsons. 2021;7:102. DOI: 10.1038/s41531-021-00241-3.
9. Mollenhauer B, Dakna M, Kruse N, et al. Validation of serum neurofilament light chain as a biomarker of Parkinson's disease progression. Mov Disord. 2020;35:1999–2008. DOI: 10.1002/mds.28206.
10. Lerche S, Schulte C, Wurster I, et al. The mutation matters: CSF profiles of GCase, Sphingolipids, α-synuclein in PD. Mov Disord. 2021;36:1216–28. DOI: 10.1002/mds.28472.
11. Simuni T, Siderowf A, Lasch S, et al. Longitudinal change of clinical and biological measures in early Parkinson's disease: Parkinson's progression markers initiative cohort. Mov Disord. 2018;33:771–82. DOI: 10.1002/mds.27361.
12. Pasquini J, Durcan R, Wiblin L, et al. Clinical implications of early caudate dysfunction in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2019;90:1098–104. DOI: 10.1136/jnnp-2018-320157.
13. Fereshtehnejad S, Zeighami Y, Dagher A, Postuma R. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal progression. Brain. 2017;140:1959–76. DOI: 10.1093/brain/awx118.
14. Ye R, Locascio JJ, Goodheart A, et al. Serum NFL levels predict progression of motor impairment and reduction in putamen dopamine transporter binding ratios in de novo Parkinson's disease: an 8-year longitudinal study. Park and Rel Dis. 2021;85:11–6.
15. De Pablo-Fernández E, Lees AJ, Holton JL, Warner TT. Prognosis and neuropathologic correlation of clinical subtypes of Parkinson disease. JAMA Neurol. 2019;76:470–9. DOI: 10.1001/jamaneurol.2018.4377.