Brasilia Parkinson Cohort: assessing clinical, neuropsychological and imaging predictors of cognitive decline in Parkinson's disease

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Abstract

The following study protocol describes the rationale and methods of a cohort with a nested case-control study, which aims to identify risk factors and predictors of cognitive dysfunction in Parkinson's disease (PD). It is a study that will follow PD every 18 months with a comprehensive neuropsychological, clinical (motor and non-motor symptoms) and imaging (Magnetic Resonance Imaging) data collection. The criteria for diagnosing mild cognitive impairment (MCI) and dementia will respect the parameters previously published by the International Working Group on Mild Cognitive Impairment, and compared with those recommended by the Fifth edition of the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association (DSM-5) and the International Parkinson's and Movement Disorders Society (MDS) criteria. We will also evaluate the neural substrate and underpinnings of PD non-motor symptoms, using advanced neuroimaging techniques, such as diffusion tensor imaging (DTI) and gray matter and white matter volumetric measurements.

Keywords

cognitive dysfunction, neuroimaging, non-motor symptoms, Parkinson disease, cohort study, dementia, mild cognitive impairment, apathy, biomarker, neuroimaging
Introduction

Parkinson's disease (PD) is the second most common neurogenerative disease in the world. Its prevalence in Brazil reaches about 3% of people over 60 years of age, according to an epidemiological study conducted in Bambuí-MG. Its pathophysiology is only partially known and involves the deposition alpha-synuclein protein fibrils, grouped as Lewy bodies. The involvement of alpha-synuclein in the pathological process led to the disease being classified as a synucleinopathy, together with multiple system atrophy (MSA) and dementia with Lewy bodies (DLB).

The trigger for the beginning of the pathological process is not yet known, but several hypotheses have been raised, based on the knowledge of the following preclinical symptoms: (1) REM sleep behavior disorder (RBD); characterized by the loss of the physiological atony of this sleep phase, manifesting as a violent movement during sleep, related to the content of dreams, and anatomopathologically associated with the degeneration of the subcoeruleus locus (more specifically, the pontine sublaterodorsal nucleus); (2) hyposmia, denoting an early involvement of the medulla and olfactory nerve by Lewy pathology; and (3) intestinal constipation, associated with synucleinopathy in the Meissner and Auerbach plexuses in the gut, and with a possible spread of the pathology to the dorsal nucleus of the vagus following a supposed upward, cranial, pathway via the vagus nerve.

One of the most relevant milestones in the knowledge of the natural history of PD came from the description of the six anatomopathological stages of the deposition of the Lewy bodies, made by the German pathologist Heiko Braak. In this description, it has been shown, in brain sections, that Lewy bodies are found earlier in the caudal portion of the brain stem, and that their dissemination in the central nervous system (CNS) would follow a caudo-cranial route. Motor symptoms, according to this theory, would be initiated in stage III of Braak, when the pathology reaches the substantia nigra and the loss of dopaminergic neurons (that innervate the basal ganglia) occurs.

More recently, there is growing recognition that PD is a systemic disease, involving both dopaminergic and non-dopaminergic neurons (serotonergic, cholinergic, noradrenergic, for example), and both CNS and extra-CNS sites. Lewy Bodies have already been identified in structures such as the sympathetic ganglia, enteric and pelvic plexus, cardiac sympathetic nerves, adrenal glands, epidermal nerves, dorsal raphe nucleus, locus coeruleus and basal forebrain nuclei (Nucleus basalis of Meynert).

In addition to this pleomorphic distribution of Lewy bodies, the disease is understood as heterogeneous, with motor symptoms (such as resting tremor, cogwheel rigidity, bradykinesia, and postural instability) and multiple non-motor symptoms. These less-recognized non-motor
symptoms still receive less therapeutic attention, although they might have an even more pronounced impact on quality of life than the classic cardinal signs
degree of these symptoms. This heterogeneity in the timeline of alpha-synuclein emergence in the CNS and in the severity of non-motor symptoms led a group of researchers to propose the existence of “non-motor subtypes” of Parkinson's disease. According to this hypothesis, there would be at least three distinct phenotypes: (1) brain stem, with late-onset hyposmia, and the predominance of sleep disorders (excessive drowsiness and RBD) and/or dysautonomia; (2) limbic, where there would be severe hyposmia or even anosmia, and the predominance of neuropsychiatric symptoms (depression, anxiety), fatigue, pain, and weight loss; and (3) cortical, with a predominance of amnestic cognitive decline, apathy, involving mainly the elderly.

Cognitive symptoms, in turn, are prevalent and bring utmost functional impact. More than 80% of PD patients develop dementia after 15-20 years of illness, and mild cognitive impairment (MCI) is identified in up to 40% of newly diagnosed patients. The most common cognitive deficits are (a) fronto-striatal, involving attention, working memory, and executive functions; and (b) posterior cortical, with changes in recognition memory and visuospatial abilities. Although the pathophysiology of this dysfunction is still unknown, individuals with a higher risk of Parkinson’s disease Dementia (PDD) appear to present an individualized subtype of PD, in which the pathology spreads more rapidly to the cerebral cortex and nuclei of the basal forebrain, associated with more diffuse cholinergic, noradrenergic and dopaminergic denervation.

Longitudinal observational studies that seek to identify the mechanism of cognitive neurodegeneration in PD have non-uniformly clustered patients, and the considerable variability in the specific operational definitions of mild cognitive impairment (MCI) is a problem yet to be solved, which limits comparability between studies and, consequently, its predictive capacity.

Understanding the progression of brain damage in PD dementia has advanced through studies using quantitative MR techniques that can measure the loss of volume of the cortical and subcortical regions over time. These methods allow the evaluation of the cortical thickness (or "cortical thinning", if longitudinal design is used), as well as the volumetric segmentation of the gray matter (GM), and measures of severity of damage to the white matter (WM), using techniques such as deformation-based morphometry (DBM), voxel-based morphometry (VBM) (MATLAB, Mathworks Inc., Natick, MA), DTI with spatial-based treatment statistics (TBSS, FSL, FMRIB Software Library Oxford University, Oxford, UK). These techniques are based on automated processing algorithms, such as FreeSurfer (Harvard University, Boston, USA), FSL and BrainVisa (IFR49, Ile de France, France).
The data generated by these MRI studies have the potential for use as specific biomarkers of diffuse or focal neurodegeneration and could help to hasten identification of groups of patients with a higher risk of cognitive decline. They would, therefore, have a predictive ability and would allow selection of a more vulnerable group of patients to be recruited into clinical trials involving drugs to prevent memory decline, for example.

Some MRI findings have been published, especially in cross-sectional studies. Beyer et al., for example, compared patients with PD and early dementia (in the first eight years of illness) or late (after eight years). In this study, patients with early PDD had more pronounced atrophy in the medial frontal gyrus and right precuneus, as well as in the left inferior parietal lobe, left superior frontal gyrus and left middle temporal gyrus. Burton et al. demonstrated, alternatively, that atrophy in the occipital lobes was strongly correlated with PDD when compared to PD without dementia, where atrophy predominated in the frontal lobes. Lateral ventricular enlargement, as well as atrophy of the corpus callosum, were also identified as potential predictors of cognitive decline in PD.

Correlations between domain-specific cognitive dysfunctions and regional cerebral volumetric measures have also been described. Camicioli et al. reported a correlation between an index of executive dysfunction and volumes of the temporal lobes and the left putamen. Ellfolk et al., in turn, showed a correlation between striatum atrophy and low performance in a phonemic verbal fluency task, as well as relationships between poor performance in the free recall of visual memory and atrophy of the right parietal cortex. Other studies have described mesial temporal lobe atrophy (hippocampal formation and entorhinal cortex) in patients with PDD, but at a lower intensity than in Alzheimer's disease. Goldman et al. showed that volumes of sub-regions of the corpus callosum (anterior, mid-anterior, central, mid-posterior and posterior) predict performance in different cognitive domains: central portion volumes would correlate with attention and working memory; while the middle-posterior portion, with distinct executive functions, language, and memory. The volume of the posterior region of the corpus callosum, in turn, would correlate with memory and visuospatial abilities. This variety of findings denotes an ongoing search for a magnetic resonance biomarker that is both feasible and reliable.

In a cohort of incident cases of PD (early stage), using DTI images processed through Tract-Based Spatial Statistics (TBSS) software, Duncan et al. demonstrated an increase in the mean diffusivity (MD), with preserved fractional anisotropy (FA), in frontal and parietal tracts of patients with reduced performance (<1.5 SD) in a semantic verbal fluency and in the Tower of London test, suggesting early loss of the frontal and parietal WM integrity in PD-MCI. Shin et al. also identified microstructural damage in WM in PD-MCI, with an increase in FA and reduction in MD in frontal areas of patients whose cognitive decline occurred more than one year after diagnosis. Price et al. showed a correlation between the reduction in cognitive processing speed, decreased FA in prefrontal WM and low volume of caudate nuclei.
Global brain volume loss rates are under study in cohorts of patients with PD and MCI. In a sample of 100 patients with PD followed by 18 months, MCI cases showed a higher rate of global brain atrophy (-1.1% ± 0.8%) and ventricular enlargement (+6.9% ± 5.2%) compared with those with intact cognition (-0.4 ± 0.5% and + 2.1 ± 4.2%, respectively), suggesting that brain volume measurements would be potential biomarkers for future clinical trials.

Most MRI studies, however, have a cross-sectional design, with biases intrinsic to the heterogeneity of the studied population, with imperfect grouping and a greater chance of combining individuals in different pathological stages or "non-motor subtypes" of DP. As such, there is still no precision as to when these changes begin, making it essential to collect longitudinal information, which would help to clarify precisely the role of structural biomarkers and resolve doubts about the potential clinical applicability of these methods.

**OBJECTIVES**

*General Objectives*

The study aims to:

(A) identify risk factors that predict a higher incidence of PD-MCI or PDD;

(B) evaluate whether there is a unique group of patients, recognized by the progression of non-motor symptoms, who are at increased risk of developing PD-MCI or PDD;

(C) investigate the association between specific patterns of volumetric changes and the microstructural architecture of cerebral WM and GM with cognitive outcomes and non-motor symptoms of PD.

*Specific objectives*

(D) use serially applied neuroimaging methods to monitor atrophy of cortical and subcortical structures potentially associated with cognitive dysfunction in Parkinson's disease;

(E) provide insights into the vulnerability of specific brain areas (in the cortical GM, subcortical nuclei, or WM tracts) as well as the "trajectory of atrophy" in specific regions;

(F) identify neuroanatomical correlates of neuropsychiatric symptoms such as depression, apathy, cognitive decline of frontal-subcortical pattern and visuospatial pattern;
(G) compare the use of different neuroimaging structural analysis techniques for the quantitative
evaluation of brain structure in the same sample;

(H) compare rates of incidence of cognitive dysfunction from the different primary operational
definitions of mild cognitive decline used for Parkinson's disease;

Materials & Methods

Study design

It is a prospective analytical observational study (concurrent cohort) with a simultaneous case-
control group (Figure 1). Recruitment of cases and controls is scheduled to begin in the first half
of 2019 and will continue until the first half of 2020.

Context

It is a local study, based in Brasilia, in the Laboratory of Neuroscience and Behavior, located at
the Institute of Biological Sciences, Department of Physiological Sciences at the University of
Brasilia. The study is carried out in collaboration with the Santa Marta Institute of Teaching and
Research (ISMEP), located in Taguatinga-DF; with the Federal University of Minas Gerais
Movement Disorders Service, located in Belo Horizonte-MG, and with N.A. Neurociência
Private Neurology Outpatient Clinic.

Participants

The study will involve a group of patients with Parkinson's disease and a group of participants
without Parkinson's disease (control group), paired by age, gender and schooling.

Recruitment strategy

Patients will be recruited directly by their attending physicians (neurologists), from an invitation
made in patient associations and through public disclosure of the study. Contact will be made
with neurologists working in the city of Brasilia, from the list of medical specialists obtained
publicly on the website of the Regional Council of Medicine, to inform about the study and
stimulate the participation of their patients. The study will be disseminated to the lay press, and
invitations to participate will be sent in the form of social media publications (Facebook, Twitter,
and Instagram, for example). The dissemination campaign will reveal the objectives of the
research and how the monitoring will be carried out, as well as the inclusion and exclusion
criteria will be exposed. It will be made clear to potential participants that their participation is
voluntary and will not imply any change in diagnostic or therapeutic decisions since it is an
exclusively observational study.

Participants in the control group will be recruited from community announcements with an
invitation to volunteer in the survey. Participants in the control group will only be recruited after
determining the demographic data of the patient group to be matched by age, gender, and
schooling.

During the study, frequent contact will be made with the participants, by telephone, e-mail or
mail, to collect information and provide results. Data will be collected to allow the future
location of patients with PD and control group participants: address, phone number, e-mail,
contacts of close friends or relatives who do not live with the subject, contact of the physician
who does the periodic clinical follow-up. This step is considered essential by the researchers, in
order to minimize the follow-up losses, which would make study conclusions unviable.

**Inclusion criteria**

Parkinson's disease patients that fulfill the following criteria will be invited to participate in the
study: (a) compliance with the diagnostic criteria of the London Brain Bank\(^4\) (the item "presence
of family history" being disregarded as a criterion for exclusion, since familial Parkinson is
relatively common and hereditary forms might be included); (b) Hoehn & Yahr disease stage
below IV\(^4\); (c) No more than eight years since the onset of symptoms of the disease.

Participants of the control group must (a) ability to understand written Portuguese (illiteracy) ;
(b) will to return for reassessment (c) paired age and schooling with the disease patients group.

**Exclusion criteria**

We will exclude recruitment candidates who present with: (a) dementia at the time of
recruitment; (b) exclusion criteria listed in the MDS Criteria for the diagnosis of Parkinson's
disease\(^5\); (c) inability to understand written Portuguese (illiteracy); (d) chronic organic disease in
an advanced stage, according to the clinical judgment of the researcher; (e) claustrophobia; (f) participants with a high chance of loss in follow-up (those planning to move out of town, unwilling to return for reassessment, health problem, or potentially fatal disease unrelated to the research question); (g) pregnancy; (h) age under 18 years old.

Participants of the control group will be excluded if they have (a) significant cognitive complaints or (b) diagnosis of neurological or psychiatric disorders (active or old) to be invited to participate in the study

**Variables**

**Primary Outcomes**

(1) Diagnosis of mild cognitive impairment (MCI), according to the criteria described below and diagnosis of PDD, according to the criteria of MDS (Emre et al., 2007), and DSM-5 for major neurocognitive disorder;

(2) A prospective cognitive decline identified by the Reliable Change Index (RCI), which is calculated as the difference between the second score and the baseline score divided by the standard error of the difference between the two scores in a specific cognitive domain. If the index is higher than 1.96, the difference is considered reliable because a change of this magnitude would not be expected only by inconsistencies of the measuring instruments. As an alternative, the modified Reliable Change Index will also be used, as suggested by MDS, where the result is considered significantly different if the RCI is higher than 1.65.

(3) Diagnosis of an apathetic syndrome (score > 13 in the Starkstein Apathy Scale and score > 2 in item 1.5 of Part I of the MDS-UPDRS scale);

(4) Diagnosis of major depression (moderate: score between 19 and 29 in BDI, severe: between 30-63 in the BDI); diagnosis of minor depression: score between 10 and 18 on BDI).

**Composite outcome:**

(5) Motor impairment I (dopamine responsive symptoms): the sum of MDS-UPDRS Part III items related to facial expression, tremor, rigidity, and bradykinesia;

(6) Motor impairment score II (non-dopamine responsive symptoms): the sum of MDS-UPDRS Part III items related to axial and speech symptoms;
**Surrogate outcome:**

Based on data from the Alzheimer's Disease Neuroimaging Initiative\(^a\), which determined the annual rates of brain volume loss for participants without neurodegenerative disease:

- the loss in total brain volume of more than 0.5% per year; annual hippocampal atrophy of more than 2.0%, of the entorhinal cortex greater than 2.5%;

**Secondary Outcomes:**

- the incidence of disabling dyskinesia or motor fluctuation, as scored in the items in part IV of the MDS-UPDRS\(^b\);
- the incidence of gait freezing, according to the score of the corresponding items in part II and III of MDS-UPDRS\(^c\);
- the incidence of clinically relevant cardiovascular and gastrointestinal dysautonomia (orthostatic hypotension) clinically relevant, according to NMSS scale scores\(^d,e\);

**Independent variables and exposures:**

Baseline demographic and cognitive data, quantitative imaging and non-motor symptoms will be analyzed as possible risk factors or predictors of cognitive impairment, based on internationally validated scales and established criteria in the literature for this assessment. To define the motor subtypes of Parkinson's disease: (a) tremor-dominant (TD); (b) postural instability- gait disorder (PIGD); and (c) mixed, the MDS-UPDRS-derived score will be used, with a cut-off >1.15 for TD and <0.90 for PIGD\(^f\).
Confounding and modifying variables

In the statistical analysis, the effect of variables will be considered through moderation and mediation analysis; a possible bias related to distinct Hoehn and Yahr stages or disease subtypes at the time of recruitment for the study will be managed by cluster analysis of non-motor data.

Diagnostic criteria for Parkinson’s disease mild cognitive impairment (PD-MCI) and dementia (PDD)

According to the criteria of the International Working Group on Mild Cognitive Impairment\(^5^8\), a patient will be diagnosed with PD-MCI if performance in cognitive tests is below the z -1.5 score, but there is still functional independence. According to the recommendations of the MDS criteria\(^4^6\), a case of MCI can also be diagnosed if there is performance between -1 and -2 standard deviations of the mean normative data; or if the cognitive decline is perceived in serial cognitive assessments with spared functional independence. The Wechler’s description system, that determines that a borderline score is between z score of -1.3 and -2.0, and an extremely low score below -2.0, will also be used.

According to the criteria of the American Psychiatric Association, 2013\(^5^9\), mild neurocognitive impairment will be diagnosed when there is a cognitive test in the range of 1 to 2 SD below the normative data, or between the 3rd and 16th percentiles.

The three criteria will have their diagnostic yield compared during the study. When using the MDS criteria, we will apply both recommendations for Level I (screening evaluation, with global cognitive screening scales only) and Level II (with full scores, and specific tests for each of five domains: attention and working memory, executive functions, memory, visuospatial function, and language).

Likewise, MDS PD-dementia diagnostic criteria\(^4^7\) will be compared with those of major neurocognitive disorder of DSM-V\(^4^8\).

Data Sources

Demographic, clinical data (disease duration, age of onset, the equivalent daily dose of levodopa, comorbidities, medications in use, family history) and neuropsychological data will be collected, with pre-specified quantitative measures being performed, with planned follow-up evaluations (Figure 1).

The following instruments will be used:

(a) Cambridge Automated Computerized Neuropsychological Assessment Battery (CANTAB)\(^6^0\);
(b) Montreal Cognitive Assessment (MoCA)\textsuperscript{61–63};
(c) Parkinson's disease- Cognitive Rating Scale (PD-CRS)\textsuperscript{64,65};
(d) Unified Parkinson's Disease Scale (MDS-UPDRS)\textsuperscript{52,66};
(e) Hoehn & Yahr Scale\textsuperscript{44};
(f) Non-motor Symptoms Scale (NMSS)\textsuperscript{54–56} (Martinez-Martin et al., 2009);
(g) Starkstein's Apathy Scale (SAS)\textsuperscript{50,67};
(h) Beck Depression Inventory (BDI)\textsuperscript{51};
(i) Hospital Anxiety and Depression Scale (HADS)\textsuperscript{68}
(j) Sleep Scale for Parkinson's Disease (PDSS-BR)\textsuperscript{69}
(k) Parkinson's disease questionnaire, reduced version (PDQ-8)\textsuperscript{70–72}, for a brief evaluation of quality of life outcomes;
(l) Parkinson's disease- Cognitive-Functional Rating Scale (PD-CFRS)\textsuperscript{73};
(m) Subtests Digits and Similarities of the Wechsler Adult Intelligence Scale, 3rd version - (WAIS-III)\textsuperscript{74,75};
(n) Trail Making Test (TMT) Parts A and B\textsuperscript{76};
(o) Symbol Digit Modalities Test (SDMT)\textsuperscript{77};
(p) Beck Scale for Suicide Ideation (BSI)\textsuperscript{78};
(q) Parkinson's Disease Impulsive-Compulsive Disorders Questionnaire – Current Short (QUIP-CS)\textsuperscript{79};
(r) Parkinson's Disease Impulsive-Compulsive Disorders Questionnaire – Rating Scale (QUIP-RS)\textsuperscript{80}.

**Accompanying intervals**

Participants will be evaluated longitudinally 3 times, on T0, T18 months and T48 months, following the detailed protocol mentioned above, and detailed in Figure 1.

**Quantitative Magnetic Resonance Imaging data**
The participants will be examined in a brief neuroimaging protocol in a high field magnetic resonance imaging device, where specific imaging sequences will be acquired for the measurement of white and gray matter volume, quantification of white matter small vessel lesions burden, subcortical white matter axonal integrity measurements, with statistical analysis using the Tract-Based Spatial Statistics (TBSS) method; and evaluation of nigrosonem-1 region.

Magnetic resonance imaging (MRI) will be performed on Philips Achieva 3.0T (Best, Netherlands), equipped with 8-channel SENSE coil, located at Santa Marta Hospital. The obtained sequences will be similar to those from the Alzheimer's Disease Neuroimaging Initiative-3 (ADNI-3) protocol, including (1) 3D-T1 TFE sequence, with FOV 208x240x256, with 1x1x1 mm resolution, TE = min full echo, TR = 2300 ms, TI = 900 ms, 2 times accelerated acquisition; (2) 3D-FLAIR sequence, with FOV 256x256x160 mm, reconstructed resolution of 1.2x1x1 mm, TE = 119 ms, TR = 4800 ms, TI = 1650 ms; (3) Diffusion Tensor Imaging (DTI), with FOV 232x232x160 mm, reconstructed resolution of 2x2x2 mm, TE = 71 ms; TR = 3300 ms, 32 directions. In addition, a high-resolution magnetic susceptibility sequence (HR-SWI) with three-dimensional acquisition will be acquired for the evaluation of the nigrosonem-1 region, according to the protocol of Schwarz et al., 2014, with the following parameters: FEEPI, TR / TE 60/30, echo train length 5, tilt angle 19°, number of slices: 50, voxel dimensions 0.55 × 0.55 × 0.70 mm, using only magnitude images.

Quantitative neuroimaging data will be analyzed in open-source software developed specifically for this purpose, such as FreeSurfer, FSL-FMRIB and volBrain.

The analysis in the FreeSurfer software, image processing involves, summarily: removal of non-cerebral tissue, automated Talairach transformation, normalization of signal intensity, extraction of the cortical surface, registration of the surface atlases, labeling of the gyrus, subcortical white matter segmentation and volume of subcortical structures according to the Desikan/Killiany atlas and determination of the thickness of the cerebral cortex. For each of the subdivided and labeled cortical regions, we intend to calculate (i) the mean cortical thickness, in mm; (ii) the total area of pial cortical surface (CSP), in mm²; and (iii) the cortical GM volume (CGMV), in mm³. The other software’s processes have specific publications with details of the method.

Voxelwise Statistical analysis will be done using Fractional Anisotropy (FA) data, using the TBSS (Tract-Based Spatial Statistics) method, which is part of the FSL application suite, University of Oxford. In the first step, FA-based images will be created by adjusting a diffusion tensor model from the raw data of the diffusion sequence (in the FDT application). Brain data will be extracted and separated from the skull cap and adjacent soft tissues by Brain Extraction Tool (BET). The FA data of all subjects,
separated by groups (PD, PD-MCI, and control group), will be aligned in a shared virtual space using the FNIRT non-linear register tool. Then, a mean FA image will be created and narrowed to represent a mean FA "skeleton", which represents the center of clustered WM tracts for each group. The FA aligned data representing each subject will be projected in this "skeleton", and the resulting data will feed the voxelwise statistics, which will compare the groups.

**Sample Size Estimate**

Defined from the sample calculation software G * Power 3 (University of Dusseldorf), using the ANOVA test with repeated measures chosen a priori, and considering inter- and intra-subject interaction for each measure, alpha = 0.05, critical F = 2.18, effect size of F = 0.25 and 4 groups for analysis (3 clinical groups - PD with normal cognition, PD-MCI and PDD, and 1 control group), with 3 total measurements performed, the minimum sample size is 60 patients with PD at the end of the study. We are considering a possible loss of 30% of the sample during follow-up, considering the three years initially planned for data collection.

**Statistical analysis**

Since multiple observations will be performed per participant, the data will be analyzed under open-source software R, using several statistical packages; the use of robust multiple regression is planned. Most of the data collected will be continuous, in order to provide further power to the statistical analysis. Categorical data, when necessary, will be dichotomized or organized in an ordinal sequence according to its nature.

The MRI data will be analyzed by generalized linear models (GLM), with pipeline entry in FreeSurfer software. As a method of controlling differences (confounding variables, subgroups, and interactions) between groups, we intend to use a clustering strategy.

It is planned to collect the data in an electronic format, with a mechanism that blocks the loading of information if there are missing or unaccounted items, as a way to avoid "missing data". Imputation of missing data will be performed by ‘mice’ R package. Missing will be treated as MAR (missing at random).

The results of robust descriptive analyzes of the sample (data central tendencies and dispersion values) will be divided by clinical group (control vs. PD vs PD-MCI vs. PDD). Inferential analyzes between groups (for example, ANOVA, repeated-measurement ANOVA and ANCOVA) will be performed using robust (resistant to outliers) methods. Bayesian statistics
The \texttt{rstan} package will be used with Hamiltonian Monte Carlo resampling in the descriptive and inferential analyzes. To control the effect of outliers from a small sample, the t-distribution will be used for the continuous data and Bernoulli for the categorical ones. Priors can be used in these analyzes, both in a non-informative (flat) and informative (obtained from the scientific literature) way.

Predictive (causal) analyzes will be investigated through path analysis with mediation and moderation (using theory-driven Mplus 8.0 models) and probabilistic graphical models (PGM) using the Bayesian Network method (\texttt{bnlearn} R package), a data-driven method.

We will use methods of Deep Learning and Machine Learning to predict atrophy of brain structures from cognitive, motor and behavioral variables and predict cognitive, motor and behavioral changes from the thickness or volume of brain structures. Several different algorithms will be tested and compared to detect that algorithm that best predicts (through its specific features) the studied outcome. It will use the \texttt{caret} R package, which integrates hundreds of other packages. The Deep Learning Toolbox, from Matlab, will also be explored, to determine the artificial intelligence algorithm with the best predictive performance.

\textit{Implications for clinical practice}

Partial and final results will be presented at international congresses on Parkinson's disease and cognitive disorders. The final results of the analysis of cohort outcome data should be published in appropriate scientific journals of neurology and cognitive neuroscience. The study aims to aid in the identification and diagnosis of Parkinson's patients at risk for cognitive dysfunction. The correct identification of this subgroup of patients will be important in the selection in future clinical trials for treatment and prevention of cognitive decline.

\textit{Evaluation by the research ethics committee}

The study will respect the declaration of Helsinki's ethical principles for medical research involving human subjects. Its protocol was submitted for analysis by the Brasilia University Center (UniCEUB) Research Ethics Committee, and approved under serial number CAEE 07073419.0.0000.0023 (technical note 3.217.185/19), registered under “Plataforma Brasil”, a national unified database for analysis of research projects involving human beings, from Brazil’s Ministry of Health. All the participants will receive complete information about the study and the data collection will only start after the signature of the consent form.
Risks and benefits assessment

No risk is foreseen for the participants of the research, since the study has a purely observational design, without intervention. The occurrence of pain, discomfort or malice is not predicted with the application of the test protocol. Attention will be given to the possibility of fatigue during testing, and the option will be offered to the participant to fragment the administration of the tests into further sessions if necessary. Among the possible benefits to participants is the provision to the attending physician of reporting gross performance on clinical and cognitive tests. The main benefit of the study, for the population of patients with Parkinson's disease, is the description of a possible biomarker of prospective cognitive decline by magnetic resonance imaging.

Financing

The study will be submitted to the analysis of funding available from support and research support agencies such as the Federal District Research Support Foundation (FAP-DF), Michael J. Fox Foundation (MJFF) and the National Council for Scientific and Technological Development (CNPq). It is already partially supported by a grant from FAP-DF [grant number 193001612/2016].

References


doi:10.1007/s00441-004-0956-9

doi:10.1111/nan.12298

doi:10.1111/nan.12303

doi:10.1007/s10072-008-0863-z


doi:10.1016/j.parkreldis.2015.09.027


doi:10.1016/j.neuroimage.2012.01.021


65. Pagonabarraga J, Kulisevsky J, Llebaria G, García-Sánchez C, Pascual-Sedano B, Gironell A. Parkinson’s disease-cognitive rating scale: A new cognitive scale specific for...
792 symptoms of Parkinson’s disease: MDS-UPDRS and NMS Scale. *Eur J Neurol.*
795 Properties of the Starkstein Apathy Scale in Patients With Early Untreated Parkinson
797 doi:10.1097/JGP.0b013e31823038f2
798 68. Faro A. Análise Fatorial Confirmatória e Normatização da Hospital Anxiety and
800 37722015032072349353
804 70. Carod-Artal FJ, Martinez-Martin P, Vargas AP. Independent validation of SCOPA–
805 psychosocial and metric properties of the PDQ-39 Brazilian version. *Mov Disord.*
807 71. Luo N, Tan LCS, Zhao Y, Lau P-N, Au W-L, Li SC. Determination of the longitudinal
808 validity and minimally important difference of the 8-item Parkinson’s Disease
811 versions of the Parkinson’s disease questionnaire in a longitudinal study. *Parkinsonism
814 impact of cognitive impairment: Validation of the Parkinson’s Disease Cognitive
816 doi:10.1016/j.parkreldis.2013.05.007
820 75. Nascimento E do, Figueiredo VLM de. WISC-III e WAIS-III: alterações nas versões
821 originais americanas decorrentes das adaptações para uso no Brasil. *Psicol Reflexão e
824 Brazilian sample on the Trail Making Test and Stroop Test. *Dement Neuropsychol.*
826 77. Spedo CT, Frndak SE, Marques VD, et al. Cross-cultural Adaptation, Reliability, and
828 doi:10.1080/13854046.2015.1093173
829 78. Beck AT, Steer RA, Ranieri WF. Scale for Suicide Ideation: psychometric properties of a
832 79. Weintraub D, Hoops S, Shea JA, et al. Validation of the questionnaire for impulsive-
834 doi:10.1002/mds.22571


Figure 1 – Brasilia Parkinson Cohort study design – The figure describes the enrollment process, along with outcome measures, clinical data, neuroimaging and neuropsychological assessments.

MRI= Magnetic resonance imaging; CANTAB = Cambridge Automated Neuropsychological Assessment Battery; MoCA= Montreal Cognitive Assessment; PD-CRS=Parkinson’s disease- Cognitive Rating Scale; TMT-A and -B: Trail Making Test Parts A and B; SDMT=Symbol Digit Modalities Test; WAIS-III=Wechsler's Adult Intelligence Scale 3rd edition; NMSS=Non-motor symptoms scale; BDI= Beck Depression inventory; BSI= Beck Suicide Ideation Scale; HADS= Hospital Anxiety and Depression Scale; PDSS-BR=Parkinson’s disease Sleep Scale, Brazilian version; RBDSQ= Rapid eye movement sleep Behavior Disorder Screening Questionnaire; SAS= Starkstein Apathy Scale; QUIP-CS= Questionnaire for Impulsive-Compulsive Disorders in Parkinson’s disease- Current Short; QUIP-RS= Questionnaire for Impulsive-Compulsive Disorders in Parkinson’s disease- Rating Scale; PD-CFRS= Parkinson’s disease-Cognitive Functional Rating Scale; MDS-UPDRS=International Parkinson and Movement Disorders-Unified Parkinson’s disease Rating Scale; DTI=Diffusion tensor imaging; TFE=turbo field echo; FLAIR=Fluid attenuated inversion recovery; PDQ-8=Parkinson’s disease Questionnaire – Short version (8-item);