UNIVERSIDADE DE LISBOA

Faculdade de Medicina de Lisboa

MULTIMODAL RESPONSE TO LEVODOPA TREATMENT IN ADVANCED AND LATE PARKINSON'S DISEASE

Margherita Fabbri

Orientadores: Professor Doutor Joaquim José Coutinho Ferreira

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Tese especialmente elaborada para obtenção do grau de Doutor em Medicina, Especialidade de Neurologia

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CONTENT

Platone (428-348 AC): "Non dovresti curare gli occhi senza curare la testa o la testa senza curare il corpo. Cosi anche non dovresti curare il corpo senza curare l'anima. (….)…una parte specifica del corpo non potrà star bene a meno che non stia bene il Tutto".

Ao meu pais, pela liberdade e apoio ilimitado,

Aos meus tres cavaileros, presente e futuro, sonhos e desafios

ABBREVIATIONS

- ADL: activity of daily living
- AE: adverse effect
- α-Syn α-synuclein
- BDI: The Beck Depression Inventory
- BMI: Body max index
- CI: Confidence interval
- CAI: continuous apomorphin infusion
- CGI-I: Clinical Global Improvement Scale
- CNS: central nervous system
- COMT inhibitors: Catechol-O-methyl transferase inhibitors
- DALYs: Disability-adjusted life years
- DBS: deep brain stimulation
- FOG: freezing of gait
- GDS: Geriatric Depression Scale
- HFS: high frequency stimulation
- HR: health-related
- HY: Hoehn and Yahr
- LCIG: levodopa-carbidopa intestinal gel
- L-dopa: levodopa
- LB: Levy Body
- LEDD: Levodopa equivalent daily dose
- LFS: low-frequency stimulation
- LHS: London Handicap Scale
- LS: late-stage
- mAIMS: modified Abnormal Involuntary Movement Scale
- MC: Motor complications
- MDS-UPDRS: Movement Disorders Society- Unified Parkinson's disease Rating Scale
- MMSE: Mini Mental State Examination
- MRI: Magnetic resonance imaging
- NA: Not applicable or not available
- NFG-Q: New freezing of gait questionnaire

- NM: neuromelanin
- NMS: Non-motor symptoms
- NMSS: Non-motor symptoms scale
- NPI-12: Neuropsychiatric Inventory test 12-items
- PD Parkinson`s disease
- PDD: Parkinson’s disease with dementia
- PDQ-8: PD questionnaire-8
- PEG: percutaneous endoscopic gastrostomy
- PIGD: postural instability and gait disorder
- QoL: Quality of life
- RCTs: randomized controlled clinical trials
- SD: Standard deviation
- S&E: Schwab & England Scale
- SN: Substantia nigra
- SNpc: Substantia nigra pars compacta
- UPDRS: Unified Parkinson`s Disease Rating Scale
- STN: subthalamic
- TD: tremor dominant
- VAS: visual analogue scale
- ZCBI: Zarit Caregiver Bur

ABSTRACT

Abstract

Parkinson's disease (PD) is a progressive age-dependent neurodegenerative disease. Life expectancy increasing and a better knowledge in PD treatment management, including the advent of device-aided therapies, are likely to increase the number of patients who can reach an advanced disease stage and eventually enter the late stage (LS) of the disease in the next decades. LSPD is a recently recognized disease stage, in which patients are severely disable and dependent on activities of daily life (ADLs) due to the presence of poor treatment responsive motor and non-motor symptoms (NMS) thus highly impacting caregiver's burden and social/health care system. Hence an operational clinical criteria to identify LSPD patients has been recently proposed suggesting adopt a Schwab and England activity of daily life score (S&E) < 50 in the MED ON condition. LSPD patients' treatment management is challenging. Treatment-related adverse effects (AEs) are frequent and few evidence in terms of phamacological and non-pharmacological treatment efficacy are available as they are barely included in clinical or research studies and even the participation into routine hospital-based visits can be an unsurmountable limit. At the same time, even if general PD disease severity milestones have been described, we do not know how LSPD patients specifically progress, if they do evolve and if there are clinical markers or biomarkers of poor outcome that could be useful to focus specific therapeutic interventions for this specific disease stage. We aimed to deeply characterize the clinical phenotype, needs along with clinical markers or biomarkers of poor outcome of LSPD patients. As levodopa (L-dopa) is the mainstay of PD treatment and a simplification of treatment regimen in later disease stages has been suggested, we also aimed to investigate the real effect of L-dopa on motor symptoms and NMS among LSPD patients, if compared to advanced stage patients. Among NMS, we focused our work particularly on speech impairment, exploring speech response to L-dopa among LSPD patients and to fine stimulation parameters adjustment, in combination with L-dopa, in advanced PD patients submitted to deep brain stimulation (DBS).

Participants were LSPD (Schwab and England ADL Scale [S&E] <50 or Hoehn Yahr Stage [HY] >3 in "MED ON" state) and advanced stage PD patients previously submitted to DBS. Cross-sectional data were obtained by means of a comprehensive clinical assessment including a L-dopa challenge test with a suprathreshold dose. A subgroup of thirteen LSPD patients underwent a neuroimaging study in order to study neuromelanin (NM) substantia nigra (SN) area changes in the latest disease stage if compared to previous ones. Automated analysis of speech were used to study the effect of a supramaximal L-dopa dose in twenty-four LSPD patients as well as L-dopa and frequency stimulation adjustment in twenty deep

brain stimulated patients. Longitudinal data were collected only for LSPD patients. Descriptive, regression and survival curves analysis were performed.

Fifty LSPD patients (female 46%) were included. Mean age was 77.5 ± 5.9 years and mean disease duration was 15.5± 6.5 years. At baseline, 76% had L-dopa-induced motor complications (MCs), mainly non-troublesome, 68%were demented, 54% had psychosis and 68% depression. Caregiver distress was high. L-dopa responsiveness was mild (18% ± 12 of improvement on MDS-UPDRS-III) and present only for appendicular signs, being tremor and rigidity the most responsive ones, while axial signs did not change. The clinical significance of this better motor response was marginal according to the Clinical Global Improvement Scale and the change in the S&E between OFF and ON state. The magnitude of L-dopa response correlated with the acute appearance of dyskinesias and the severity of MCs. After one-year, 20% of the patients were dead, 18% institutionalized in nursing home and 6% passed to a HY 5. MDS-UPDRS-motor mean score worsened 7.2 ± 10.3 points, corresponding to a 15.7% (±23.0) increase, with no difference between tremor-dominant versus akinetic-rigid phenotype or PD patients with/without dementia (PDD/non-PDD) at baseline. However, there was heterogeneity between patients in terms of disease progression, as 12 patients (37.5%) had a motor deterioration ≤ 3 points and 14 (43%) ≤ 5 points with concomitant worsening of the MDS-UPDRS-II (Motor Aspects of Experiences of Daily Living), of 2.1±4.1. Conversely, eleven cases (32%) did not deteriorate and, in fact, 10 of these improved between 1-6 points at the MDS-UPDRS-III. Overall NMS worsened, mostly in cognition/mood, urinary and gastrointestinal domains. Conversely, MCs improved despite similar L-dopa equivalent dose. Functional independence and quality of life worsened. Dysphagia severity at baseline predicted a poor combined outcome (death, being institutionalized or developing HY 5) (Hazard ratio 2.3, 95% CI 1.12- 4.4; p = 0.01) or death alone (Hazard ratio of 2.9, 95% CI 1.12- 8.6, p=0.04), whereas magnitude of L-dopa response of LSPD patients did not.

SN area evaluated by NM-sensitive magnetic resonance imaging (MRI), resulted able to differentiate LSPD patients from both *de novo* PD patients and controls, though not founding statistical differences between LSPD patients and patients with two-five year disease duration.

Performing an indirect comparison of the effect of L-dopa on motor symptoms and NMS among twenty LSPD patients and twenty-two, not-matched, advanced PD patients, a milder

response on motor symptoms (11% vs. 37% of improvement on MDS-UPDRS-III) and an absence of response on NMS, namely anxiety, fatigue and pain, were found among LSPD patients, with concomitant higher frequency of drug-related AEs. Indeed orthostatic hypotension (OH) or drowsiness occurred among 35% of LSPD patients versus 13% of advanced PD patients, who still presented a benefit from L-dopa intake on pain and anxiety, while fatigue did not change. Scales applicability and blood pressure assessment while standing resulted challenging among LSPD patients with consequent missing data on depression, anxiety, pain and OH identification and possible underestimation of those symptoms. No effect of L-dopa was found on speech and voice by means of both automated analysis and clinical evaluation in LSPD patients. Respiratory support for speech and voice stability were the most affected speech and voice features among LSPD patients. Among axial symptoms, speech seemed to be the most L-dopa unresponsive one. Speech unresponsiveness to L-dopa was confirmed also among subthalamic (STN)-DBS treated patients with both mild and severe dysarthria, at least in combination with stimulation. Conversely, PD patients with severe dysarthria under chronic STN-DBS treatment showed a benefit of lowering frequency of stimulation from 130 Hz (High frequency stimulation [HFS]) to 60Hz (low frequency stimulation [LFS]), with concomitant increment of voltage, in order to keep constant the total energy delivered. Indeed speech intelligibility and articulatory diadochokinesis presented an acute improvement passing from HFS to LFS, as assessed by automated speech analysis and such a benefit, when present and clinically meaningful, lasted during six months with no motor worsening, though requiring medication adjustment.

The present study provides further evidence to better delineate a recently recognized and poorly described PD stage. An extensive cross-sectional and longitudinal observation is proposed. LSPD patients clearly differ from previous stages in terms of both clinical features, needs, therapeutic response and drugs' tolerability profile. Over one year, a heterogeneous disease progression of motor symptoms is still present and it seems even steeper if compared to previous stages, while functional independence globally worsened. As well as mild motor improvements are still possible with treatment adjustment, it is also possible to identify a clinical phenotype of LSPD patients who are likely to have a better response to L-dopa if compared to the other ones. Clinical assessment and therapeutic interventions for swallowing problems should be a priority. PDD or living in a nursing home remain other indicators of poor outcome. In the next few years the number of LSPD patients

who have been previously submitted to device-aided therapies is expected to increase, bringing new clinical scenarios, such as the fine parameters adjustment of invasive treatment for challenging motor and NMS and the difficult management or eventual interruption of those treatments among elderly and frail LSPD patients.

Overall, future research and fund allocations should be specifically oriented on LSPD patients, usually not included or considered in clinical trials or research studies, and on L-dopa not-responsive aspects and caregivers' needs.

Key words: Parkinson's disease; late-stage; advanced stage; levodopa; disease progression;

RESUMO

A doença de Parkinson (DP) é uma doença neurodegenerativa cuja incidência aumenta com a idade. É antecipado que nas próximas décadas, com o aumento da esperança de vida e a melhoria dos cuidados de saúde, incluindo o acesso a tratamentos mais invasivos, ocorra um aumento do número de doentes que vão chegar a fases mais avançadas da doença, incluindo os recentemente descritos estádios tardios. Nesta fase da doença, os doentes apresentam-se incapacitados e dependentes para as atividades de vida diária em virtude da presença de sintomas motores e não motores. Estes sintomas respondem pouco aos tratamentos disponíveis, acabando também por afetar os cuidadores e terem impacto no serviço social e sistema de saúde. Recentemente foi proposto um ponto de corte na escala de Schwab & England (independência funcional nas atividades de vida diária) de 50% como critério clínico operacional para identificar doentes na fase tardia de doença. É consensualmente reconhecido que o tratamento de doentes com DP na fase tardia é difícil. A ocorrência de efeitos adversos relacionados com os tratamentos também é frequente. A evidência científica de eficácia de intervenções farmacológicas ou não farmacológicas nesta fase da doença é baixa, sendo estes doentes muito frequentemente excluídos de estudos clínicos. De igual forma, devido à incapacidade também deixam de conseguir comparecer nas consultas hospitalares.

Apesar de serem bem conhecidos os problemas que condicionam incapacidade nos estádios mais avançados da doença, não é ainda bem conhecido como a doença progride na fase tardia e se existem marcadores clínicos ou biomarcadores de progressão de doença, úteis para serem utilizados na avaliação de possíveis intervenções terapêuticas.

O objectivo do nosso estudo foi caracterizar as manifestações clínicas, as necessidades, e os marcadores clínicos ou biomarcadores de pior prognóstico na fase tardia da DP.

A levodopa é o medicamento padrão para o tratamento da DP e uma simplificação no esquema terapêutico da sua utilização na fase tardia da DP foi recentemente sugerido. Em consequência, o nosso objectivo foi também investigar o efeito clínico da levodopa nos sintomas motores e não motores em doentes em fase tardia, comparado com doentes em fase avançada. Entre os sintomas não motores, foi dada maior atenção à alteração da fala, investigando o efeito da levodopa sobre a fala em doentes em fase tardia e o efeito de um ajustamento dos parâmetros de estimulação cerebral profunda (ECP), em associação com a levodopa, em doentes em fase avançada submetidos a ECP do núcleo subtalâmico (NST).

Foram incluídos doentes em fase tardia (escala de Schwab & England ADL <50% ou escala de Hoehn & Yahr >3 durante o efeito da levodopa, MED ON) e doentes em fase avançada, previamente submetidos a ECP do NST. Procedeu-se a uma avaliação transversal dos doentes utilizando uma avaliação clínica detalhada, incluindo um teste agudo à levodopa com dose supra-máxima. Um subgrupo de doentes em fase tardia foi submetido a um estudo de neuroimagem cerebral por ressonância magnética para avaliar a área de sinal da neuromelanina na substância nigra e comparar os resultados com estádios mais precoces da doença. Uma análise automática da fala foi realizada para avaliar o efeito da levodopa em 24 doentes em fase tardia e também para avaliar o efeito da um ajuste da frequência de estimulação, em associação com a levodopa, em 20 doentes em fase avançada submetidos a ECP do NST. Uma avaliação prospetiva dos doentes foi realizada para os doentes em fase tardia. Foi efetuada uma análise descritiva dos dados e aplicados modelos de regressão e curvas de sobrevida.

Cinquenta doentes em fase tardia (46% mulheres) foram incluídos. A idade média foi 77.5 ± 5.9 anos e a duração média da doença de 15.5± 6.5 anos. Na primeira visita, 76% dos doentes apresentavam complicações motores relacionadas com a levodopa, principalmente não incómodas, 68% apresentavam critérios de demência, 54% apresentavam alucinações e 68% encontravam-se deprimidos. A sobrecarga dos cuidadores foi elevada. A reposta à levodopa foi ligeira (18% ± 12 de melhoria na escala MDS-UPDRS-III) e detetável só para sintomas apendiculares, sendo o tremor e a rigidez os que responderam melhor, enquanto os sintomas axiais não apresentaram alterações. A relevância clínica desta resposta foi marginal de acordo com a Escala de Impressão Clínica Global e com os valores da escala de Schwab & England em MED ON e MED OFF.

A magnitude da resposta à levodopa revelou uma correlação com o aparecimento das discinésias e a gravidade das complicações motoras. Após o período de um ano, 20% dos doentes tinham falecido, 18% foram institucionalizados e 12% passaram a ter um HY de 5.

O valor médio da MDS-UPDRS-III agravou-se em 7.2 ± 10.3 pontos, o que corresponde a um aumento do 15.7% (±23.0), não tendo sido documentada uma diferença entre os doentes com fenótipo tremórico e os doentes com fenótipo acinético-rígido ou os doentes com ou sem demência, no momento da inclusão no estudo. Em contraponto, ocorreu uma progressão heterogênea da doença, sendo que 12 doentes (37.5%) apresentaram um agravamento motor ≤ 3 pontos e 14 (43%) ≤5 pontos, com um concomitante agravamento

do MDS-UPDRS-II (aspetos motores das atividades de vida diária) de 2.1±4.1. Onze doentes mantiveram-se estáveis, e dez doentes melhoraram de 1 a 6 pontos na MDS-UPDRS-III. Globalmente os sintomas não motores agravaram, tendo ocorrido um agravamento dos domínios cognitivo/humor, dos problemas urinários e gastrointestinais.

Em contrapartida, as complicações motoras melhoraram, apesar de a dose diária de levodopa ter-se mantido estável. O nível de dependência funcional e a qualidade de vida agravaram.

A gravidade da disfagia na primeira visita previu a ocorrência do resultado combinado de morte, institucionalização ou mudança para um estádio HY de 5 (hazard ratio 2.3, 95% CI 1.12- 4.4; p = 0.01) ou unicamente do resultado morte (hazard ratio 2.9, 95% CI 1.12- 8.6, p=0.04), enquanto a magnitude de resposta à levodopa não constituiu um fator de prognóstico significativo.

A área de neuromelanina da substância nigra diferenciou doentes com DP em fase tardia de doentes de novo e controlos, mas não foi encontrada uma diferença estatisticamente significativa entre doentes em fase tardia e doentes com 2 a 5 anos de doença.

Foi efetuada uma comparação indireta entre o efeito de um teste agudo com levodopa em doentes em fase tardia (20 doentes) com doentes em estádio avançado (22 doentes) e evidenciada uma resposta ligeira nos sintomas motores (11% versus 37% de melhoria na escala MDS-UPDRS-III) e uma ausência de efeito sobre os sintomas não motores, como ansiedade, dor e fadiga, nos doentes em fase tardia que apresentaram também mais efeitos adversos. A hipotensão ortostática (HO) e a sonolência ocorreram em 35% dos doentes em fase tardia em comparação com 13% dos doentes em fase avançada que apresentaram um benefício na dor e na ansiedade, mas não na fatiga.

A aplicação de escalas e a avaliação da pressão arterial em pé revelou-se difícil de realizar nos doentes em fase tardia, resultando numa maior falta de dados sobre a depressão, ansiedade e a presença de HO, com possível subavaliação desses sintomas.

A levodopa não induziu melhoria na fala e voz em doentes em fase tardia, seja através de analises automáticas seja de acordo com a avaliação clínica. O suporte respiratório da fala e a instabilidade da voz foram as caraterísticas da fala mais afetadas em doentes com DP em fase tardia.

Entre os sintomas axiais, a fala foi o que respondeu pior ao tratamento com levodopa. A ausência de benefício na fala depois da toma de levodopa foi encontrada também em doentes submetidos a ECP do NST com disartria ligeira ou grave, pelo menos em associação com a estimulação. Em contrapartida, doentes com disartria grave com ECP cronica do NST, podem beneficiar da redução da frequência de estimulação de 130 Hz (alta frequência) até 60 Hz (baixa frequência). Contudo, foi necessário aumentar a voltagem para manter constante a energia liberada. A inteligibilidade da fala e a diadococinesia articulatória apresentaram uma melhoria, na passagem da alta a baixa frequência de estimulação, de acordo com analises automáticas da voz. Este benefício, quando presente e clinicamente relevante, manteve-se durante seis meses sem agravamento motor, mas necessitando de ajuste na medicação oral.

O nosso estudo contribui com dados adicionais para a definição de fase tardia de DP, ainda pouco estudado. Uma avaliação detalhada transversal e prospetiva foi realizada. Os doentes com DP em fase tardia são claramente diferentes em termos clínicos, de necessidades, resposta ao tratamento com levodopa e tolerabilidade aos fármacos. A doença progride de forma heterogenia ao longo de um ano, de forma ainda mais intensa que em estádios anteriores e em paralelo com um agravamento global da independência funcional. É possível induzir pequenas melhorias em termos motores com o ajuste da medicação. Ao mesmo tempo foi possível identificar um fenótipo de doentes em fase tardia que tem maior probabilidade de responder à levodopa.

A avaliação clínica e as intervenções terapêuticas para a disfagia são uma prioridade nesta fase da doença. Demência e institucionalização continuam a ser outros indicadores de pior prognóstico. Nos próximos anos o número de doentes com DP em fase tardia que foram previamente submetidos as terapêuticas invasivas nas fases avançadas vão aumentar e um novo perfil de doentes vai surgir. Os neurologistas vão ter que ajustar os parâmetros das terapias de fase avançada no tratamento de sintomas motores e SNM mais complexos e aprender a gerir estas terapias invasivas, incluindo a possível interrupção em doentes com DP em fase tardia, idosos e frágeis.

Na nossa opinião será necessário alocar recursos e realizar estudos dirigidos à população de doentes com DP em estádios tardios (em geral não incluídos em ensaios clínicos), aos sintomas que não respondem à levodopa e às necessidades dos cuidadores.

Palavras-chave: doença de Parkinson; estádio tardio; estádio avançado; levodopa; progressão de doença;

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INTRODUCTION

Parkinson's disease

PD is an age-related neurodegenerative disorder, characterized by progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), particularly in its lateral ventral tier, associated with Lewy pathology. [1-3] PD is classified as a synucleinopathy, as α-synuclein (α-Syn), a presynaptic neuronal protein, is the major constituent of Lewy bodies (LBs), which are a pathological hallmark of PD. [4] Lewy pathology is also found in extranigral regions of the central nervous system (CNS), such as the pons, basal forebrain, limbic cortex or higher order association cortices and additionally in the peripheral autonomic nervous system, thus affecting not only the dopaminergic system but also the cholinergic, noradrenergic and serotonergic ones.[5]

PD is the second most common age-related neurodegenerative disorder after Alzheimer's disease. [6] The Global Burden of Disease Study estimated that in 2015 there were 6.2 million people affected by PD, which resulted in about 117,400 deaths worldwide. PD's mean age of onset is about 65 years but prevalence increases steadily with age. [7] Moreover it was estimated that the number of individual over age 50 with PD was between 4.1 and 4.6 million in 2005 and will double to between 8.7 and 9.3 million by 2030.[8]

PD, as other neurodegenerative diseases, is a complex disorder occurring from the interplay between genetic, environmental, nutritional and other factors, together with aging.[9] In fact, although mutations in specific genes have been shown to participate in the etiology of PD, the genetic accounts for only 5–10% of all PD cases, suggesting an additional role for exogenous or environmental factors in the etiopathogenesis of the disease. Among environmental factors, there are suggestive evidences for pesticides increasing PD risk, particularly for insecticides, than for any specific compound,[9] while smokers and partly coffee drinkers have a lower risk of PD.[10] Even if the precise molecular mechanisms causing neuronal loss are still not fully understood, several pathways and mechanisms involved in PD pathophysiology have been identified: a) α-Syn aggregation[11]; b) Prion-like cell-to-cell transmission of α-Syn, following a rostro-caudal gradient throughout the enteric nervous system, via the vagal nerve and olfactory tract, to the SN and further areas of the CNS (the gut-brain axis)[12, 13]; c) Mitochondrial dysfunction intimately linked to dysfunction of axonal transport, nigral dopaminergic neurons vulnerability and oxidative stress[14, 15]; d)

Impairment of autophagy[16]; e) Neuroinflammation, with intense astrogliosis and microgliosis that may be associated with abnormal corticostriatal plasticity.[17]

The classical motor features of PD are an asymmetrical bradykinesia, lead pipe type rigidity and a 4-6 Hz pill-rolling rest tremor, as well as postural instability later in the disease course. [3, 18] However, non-motor symptoms (NMS) such as dysautonomia, pain, sleep disturbance, depression, psychosis and dementia are now well established features of PD and they classically increase in frequency and severity in later stages of disease. [19] With no disease-modifying therapies available, PD remains an incurable neurological condition. [20, 21] Levodopa (L-dopa) treatment is the mainstay therapy and the gold standard for the control of disease motor symptoms. [22, 23] Almost all patients will eventually take L-dopa at some stage during their illness. Yet, L-dopa therapy has introduced an additional source of features into the natural evolution of PD through its potential to induce involuntary movements as well as motor response fluctuations. [24, 25] The high prevalence of L-dopa related motor complications (MC) and NMS make very difficult the achievement of a satisfactory symptomatic control once patients reach a more advanced disease stage. [26] Moreover, the disease continues to progress, and non-dopamine-responsive symptoms such as cognitive dysfunction and imbalance become more prominent and lead to long-term disability.[27]

Parkinson's disease staging

Neurodegeneration in PD likely begins years or decades before full PD diagnosis can be made and the existence of a pre-motor PD phase is now universally recognized. [28-30]An accepted definition of PD staging is still lacking, but the natural history of PD can be divided into a an Early stage, an Intermediate or Moderate stage and an Advanced stage, according to the presence and severity of motor symptoms, the presence and severity of MC and the physical independence of the patients. Recently, a definition of a later PD stage has been also proposed (see next paragraph). Early PD stage, in turn, can be divided into the following three stages: I) Preclinical: neurodegeneration is present but without measurable symptoms or signs, thus requiring biomarker diagnosis. II) Prodromal: symptoms/signs are present, but they are insufficient to diagnose clinical PD; III) Clinical: this implies the presence of parkinsonism (bradykinesia with fatiguing/decrement plus one of rest tremor or rigidity). The

importance of the prodromal PD phase has been universally recognized also by the recently elaboration of the Movement Disorder International Society (MDS) criteria for prodromal PD, currently used only in research field, due to the lack of effective neuroprotective treatment.[31]

The clinical onset of PD is defined by the appearance of motor symptoms. According to the recently proposed MDS criteria for PD diagnosis, the first essential criterion is the presence of parkinsonism, which is defined as previously mentioned, as bradykinesia, in combination with at least one of rest tremor or rigidity. [32] Supportive criteria, absolute exclusion criteria and red flags, should be also considered in order to define a "clinically defined PD" or a "clinically probably PD". [32] The onset of motor signs is typically asymmetric. Over time, symptoms progress to the other side and affect also axial domains. Interestingly, postural instability is not part of the recent "MDS PD criteria" as its presence early in disease suggests an alternative diagnosis as it often occurs in later PD stages. [32] Although the definition of different phenotypes of PD is based on motor symptoms, NMS are manifested from the early start of PD affecting all non-motor domains.[19, 33]

Clinical characteristics, response to therapy and disease course could be very different among PD patients, accordingly to clinicopathologic phenotypes and age at disease onset. Indeed, patients with young-onset (YO) PD initially presented more often with rigidity and dystonia, had a higher frequency of L-dopa-related MC in spite of an excellent response to L-dopa than those with late-onset PD, who presented more often with the postural instability and gait disorder dominant (PIGD) pattern and a slower disease progression. [34-36] On the contrary patients with a tremor dominant (TD) clinical picture at onset may have a slower disease progression, being also identified as "benign tremulous parkinsonism" with predominant rest tremor, mild non-tremor motor signs, absence of gait disorder, and mild progression of parkinsonism other than tremor despite many years of disease. [36] Overall PD motor progression is non-linear, more pronounced in patients early in the disease course and with lower motor impairment. Reported annual increase of motor impairment has been estimated around 2.4 points in the UPDRS-III and 2.2 in UPDRS-II within the first five years of disease with standardized annual progression rate ranging from 2.4% to 7.4% in intermediate

disease. [37, 38] A slower rate of progression has been reported in more advanced stages of PD. [37]

The emergence of L-dopa-induced MC is a landmark in the clinical progression of PD. The appearance of L-dopa related MC, or at least L-dopa troublesome MC, defines the beginning of the advanced PD stage.[39, 40] The frequency of MC can reach 40% of patients after 4-6 years of L-dopa treatment. [24] The control of MC remains an unmet clinical need. MC are a major source of disability for patients and caregivers, they are associated with a poor quality of life (QoL) and with a decreased independence of patients for the activities of daily living (ADLs).[41, 42] Troublesome MC usually require a complex drug regimen and are the major clinical indication for device-aided therapies. [43, 44] Besides MC, PD patients in advanced stage also manifest several NMS and axial motor features resistant to L-dopa such as postural instability, falls and dysphagia, which increase in frequency and severity with longer disease duration. [27] An alternative definition of advanced PD patients adopts the Hoehn and Yahr scale (HY), identifying PD patients in a 4 or 5 HY during the medication (MED) OFF period. [45, 46] The HY scale, developed in a pre-L-dopa era, is still the most widely used tool to stage severity of parkinsonism, in spite of recognized limitations as a measure of disease progression. [47, 48] Indeed, it is based on the concept that the severity of parkinsonism depends mainly on the presence of bilateral symptoms and compromise of gait and balance. Moreover, it is heavily weighted towards postural instability and lower limbs involvement, though not considering the presence of NMS or MC, which are likely associated to disease progression. [49]As a result, patients of different disease severity can be included in the same HY stage, which become clinically heterogeneous. [48] Finally is it increasing evident that the common concept of advanced PD is a “large umbrella” that includes a wide spectrum of patients that can be characterized by heterogeneous patterns of MC, NMS and several grade of physical dependence. Indeed patients owing very different clinical characteristics fall in the advanced definition, but some may do not fulfill the characteristic of the advanced phase.

Late-stage Parkinson's disease concept

In the last decade, it has been observed that a small subset of patients with advanced-stage PD progress to a later phase of disease, clinically discernible from the previous one. An increase in life expectancy[50] and a better clinical management of PD are likely the main cause of the increased number of patients with a more prolonged disease course. Moreover taking into consideration that ageing is the strongest risk factor for PD, the prevalence of PD will increase substantially in the next two decades. [7, 51]

In this later stage the cardinal PD motor symptoms are quietly changed as patients are usually characterized by severe bradykinesia with reduced or absent rigidity. [52-54] Disability from MC is classically reduced, because these complications attenuate naturally, either for L-dopa treatment reduction or in response to device-aided therapies. [54, 55] Indeed, the prevalence of L-dopa-related MC of this late phase is very variable, in agreement with different studies, ranging from 48% to 100% for motor fluctuation and from 42% to 100% for dyskinesias, but significantly lowering if considering troublesome fluctuations (10%-36%). [54] Thus disability in the later stage is dominated by a cluster of variables that consists of NMS as cognitive impairment, psychosis, depression, daytime sleepiness, autonomic dysfunction[53], and axial symptoms classically resistant to L-dopa and resulting in a "late" phenotype whose clinical features do not really fit with the common concept of advanced stage, classically characterized by disabling MC.[52, 56 57] The Sydney cohort study reports outcomes among 30 patients surviving until 20 years of follow-up, showing as falls, freezing, dementia and moderate dysarthria were each observed in over 80%, hallucinations, excessive daytime sleepiness and urinary incontinence were each experienced by more than 70%, and choking occurred in 48%. [56] Coelho and colleagues reported as LSPD patients handicap is mostly driven by the presence of dementia, behavioural complaints and the severity of non-dopaminergic motor features. [53]

Indeed four principal disability milestones, defined as the symptoms of disease advancement that are likely to require additional medical attention,[58] have been also identified to precede death by around 5 years and they are: visual

hallucinations (5.1 years), falls (4.1 years), dementia (3.3 years) and institutionalization (3.3 years).[59] Age at disease onset seems to markedly determine disease clinical characteristics, the pattern of response to L-dopa and how long a patients will be disease severity milestones free but once reached the late phase the clinical picture seem to be quite homogeneous both from a clinical and from a neuropathological point of view. [58] The term "late-stage" was recently proposed in order to identify PD patients who are highly dependent on caregivers for ADL and own treatment-resistant motor symptoms or NMS. [52] To better characterize the grade of disability in ADL, Coelho and Ferreira has proposed the use of the Schwab and England activity of daily life score (S&E), considering also the limit of the HY in this late phase due to its motor-oriented base.[52] S&E is an easy administrable 100-point questionnaire in which 0% denoted a bedridden or vegetative state and 100% a normal ability with complete independence.[60] It correlates with UPDRS and its sensitivity increases with higher HY stages.[61] The proposed cut-off for defining a LSPD patient is a score on the S&E of less than 50% during "MED ON" state. A score of 50% corresponds with the patient requiring help with half of their chores and experiencing difficulty with all activities. Overall, LSPD stage is characterized by patients dependent on caregivers for their activities of daily living, even under the best L-dopa benefit.

The number of LSPD patients is expected to increase in the next future, carrying a higher burden of disease for patients, caregivers, the healthcare and social security systems. [8, 62, 63] Very few studies have addressed the characteristics of LSPD, probably due to the relatively recent appearance of this phenotype and the difficulties in recruiting these very disabled patients. Indeed, we can consider LSPD an orphan population whose clinical phenotype and management have not been systematically analyzed yet. [54, 64]

Management of late-stage Parkinson's disease

Several burdens can be identified in the treatment of LSPD patients, which make the management of those patients particularly challenging. [52] Overall, few randomized controlled clinical trials (RCTs) specifically addressed LSPD patients as a target population. Hence, scarce systematic data exist for the treatment of motor and NMS of LSPD patients and treatment recommendations regarding these patients are frequently based on expert opinions and good clinical practice. [64] So far, no recognized prognostic factors have been identified for this orphan population in order to alert clinicians on clinical crucial problems to which specific treatment interventions should be addressed. Moreover, recommended assessment tools for these highly disabled patients are still lacking and caregivers still have a marginal role when considering possible therapeutic interventions. [65]

As previously mentioned, LSPD clinical picture is characterized by severe dependence, with major limitations even for minimal postural transfers and severe NMS, which all together severely impact patients and caregiver's QoL. [52, 62]

The management of NMS represents an emerging unmet need in the treatment of patients with PD throughout all the disease course and above in the later stages as current therapies for NMSs in PD are limited. [19 ,64] Few pharmacological interventions have been considered "clinically useful" by the MDS Evidence-Based Medicine Review for the treatment of few NMS frequently present in LSPD patients, such as dementia, psychosis and sialorrhea, that can be treated with rivastigmine, quetiapine and botulin toxin injections, respectively. [66] Recently a 5-HT_{2A} inverse agonist, pimavanserin, has been also approved in the United States for the treatment of dopamimetic-induced psychosis in PD. [67] Several non-pharmacological interventions have been also investigated for the treatment of poor L-dopa responsive symptoms. [68] Even if the beneficial effect of physical therapy and, partly of occupational therapy, has been shown on physical performance, Qol and abilities in ADL, no RCTs specifically addressed those interventions to LSPD patients. [68] Regarding swallowing problems, only one small RCT found little evidence to support the effect of a video-assisted swallowing training. [69]

Because of the multidimensional nature of PD, virtually every patient may need an individualized management program. [70] Multidisciplinary care approaches have been shown to have a positive benefit on disability and QoL of PD patients. [70] However, their effect if compare to usual care was not clearly clinically relevant for patients, probably due to

methodological limitations of those studies. [10, 71] Moreover no studies on multidisciplinary care were specifically addressed to LSPD patients and cost-related or feasibility evaluations throughout different countries and health care systems still need to be investigated. Recently the relevance of a palliative care approach, intended as an holistic approach to the patient, including life experiences, patients' and family caregivers' QoL, the optimization of symptomatic management, and the establishment of an open communication with the patient, family and an interdisciplinary team, has been pointed out, even from the very beginning of the disease. [72, 73] Given the complex clinical picture of LSPD patients, an implementation of integrated neurological and palliative care interventions is desirable in this later disease phase. [72]

Taken as a whole, the landscape of LSPD management is a list of unmet clinical needs and unsolved burdens for patients, caregivers and clinicians.

A final consideration should be made on the use and role of L-dopa in LSPD patients. Indeed, L-dopa is still the gold standard of PD treatment[22, 23] and it can have a favourable safety profile in the elderly population, if compared to other antiparkinsonian medication. [74, 75] Few data have shown that neurologists tend to simplify the drug regimens in PD patients in late stages[52, 54], due to the side effects of antiparkinsonian drugs and / or an apparent loss of benefit from L-dopa. Indeed a previous study demonstrated as up to 40% community-dwelling LSPD patients are undertreated.[76] However, it is still open to debate whether this apparent loss of benefit from L-dopa is real, or alternatively it is the result of downgrading the dosage of L-dopa due to the occurrence of side effect and which is the real response to L-dopa among LSPD patients.

AIMS OF THE STUDY

The present study aimed to investigate disease progression and therapeutic management of advanced and late-stage PD patients. The purposes of the study were:

1. To investigate clinical and neuroimaging markers of disease progression in late-stage PD patients;
2. To study the response of motor symptoms to L-dopa in LSPD patients;
3. To study the response of NMS to L-dopa in LSPD patients;
4. To study the response of speech to L-dopa in LSPD patients;
5. To investigate the effect of stimulation parameters adjustment in combination with L-dopa on dysarthria in PD patients under chronic subthalamic deep brain stimulation (STN-DBS);

CHAPTER 1: Disease progression in late-stage Parkinson's disease

Clinical and neuroimaging features

Dysphagia predicts poor outcome in late-stage Parkinson's disease

Abstract

Background: Few data exists on the rate of clinical progression for Parkinson's disease (PD) patients who have entered a late stage of the disease.

Objective: Study the clinical progression of a late-stage PD (LSPD) population over one year follow-up.

Methods: 50 LSPD patients (Schwab and England ADL Scale <50 or Hoehn Yahr Stage >3 in MED ON) underwent an extensive clinical assessment at baseline and after one year and an acute levodopa test at baseline.

Results: Mean age of LSPD patients (female 46%) was 77.5 ± 5.9 years and mean disease duration was 15.5± 6.5 years. At baseline, 76% had levodopa-induced motor complications (MC), usually non-troublesome, 68% were demented, 54% had psychosis and 68% depression. Caregiver distress was high. L-dopa responsiveness was mild (18% ± 12 of improvement on MDS-UPDRS-III). After one-year, 20% of the patients were dead, 18% institutionalized and 12% passed to HY 5. MDS-UPDRS-motor mean score worsened 7.2±10.3 points although there was heterogeneity between patients, and there was a global worsening of non-motor symptoms, mostly in cognition/mood, urinary and gastrointestinal domains. Nevertheless, MC improved despite similar levodopa equivalent dose. Functional independence and quality of life worsened. Dysphagia severity at baseline predicted a poor outcome (death, institutionalization or HY 5) (Hazard ratio 2.3, 95% CI 1.12- 4.4; p = 0.01), whereas magnitude of L-dopa response of LSPD patients did not.

Conclusions: LSPD patients still present a significant, although heterogeneous, motor and non-motor progression over 1 year. Dysphagia severity predicts the occurrence of additional disease severity milestones and its management must be prioritized.

Introduction

Progression in Parkinson's disease (PD) seems to be exponential in its later stages.[52] Indeed, a number of advanced PD patients enter a later stage when motor and non-motor symptoms (NMS) such as falls and dementia rapidly aggravate, causing a major impact on the health status and independence of patients. [52, 54] Nonetheless, scarce data exists on the rate of clinical progression and prognostic factors for patients who have already entered a late disease stage.[55, 56] Equally, uncertainty exists whether the magnitude of levodopa (L-dopa) responsiveness is a prognostic factor in late-stage PD (LSPD).

Our aim was to study the clinical progression and response to L-dopa in a LSPD sample over one-year follow-up.

Patients and methods

Primary objective

To study the clinical progression of a LSPD population over one year follow-up.

Secondary objective

To study the response of LSPD patients to a suprathreshold dose of L-dopa.

Study design and patients recruitment

We performed a cross sectional study and a prospective cohort study. Patients were consecutively recruited from the Movement Disorders outpatient clinic of a tertiary university hospital. Idiopathic PD patients, according to the UKBB criteria,[77] were included in the study if they had a Schwab and England score (S&E) < 50%[60] or a Hoehn & Yahr Stage (HY) >3 in MED ON. LSPD patients were assessed at baseline and at 1 year follow-up (range 12-15 months). The Local Ethical Committee approved the study and all patients provided informed consent.

Patients' assessment

At baseline, patients underwent an extensive clinical assessment including a challenge test with a supra-maximal dose of L-dopa. Details of L-dopa challenge test were previously reported.[78, 79] Overall, during both "MED OFF" and "MED ON" conditions the following parameters were evaluated: a) motor performance using the MDS-UPDRS part III scale,[49]

the Modified Abnormal Involuntary Movement Scale (mAIMS)[80] and the HY stage; b) the change of specific NMS: blood pressure (BP) measured in supine and 3 minutes after standing, presence of orthostatic hypotension (OH), pain and fatigue using a visual analogue scale (VAS-p and VAS-f, respectively). L-dopa equivalent daily dose (LEDD) was calculated according to standard conversions.[81] Clinical phenotypes, i.e. akinetic-rigid (AK) and tremor dominant (TD), were defined in concordance with clinical history. NMS were evaluated using the MDS-UPDRS part I, the Non-Motor Symptoms Assessment Scale for PD (NMSS)[82], the Neuropsychiatric Inventory test (NPI) 12-items and the Geriatric Depression Scale (GDS) MDS-UPDRS parts II and IV assessed the impact of motor symptoms on activities of daily life (ADL) and L-dopa-induced MCs, respectively. Diagnosis of PD with dementia (PDD) was made in agreement with the Level I algorithm of the MDS Task Force recommendation for probable PDD diagnosis.[83] Quality of life (QoL) and health-related (HR)-QoL were assessed using the PD questionnaire 8 (PDQ-8) [84]and the Visual Analogue Scale of the Euro-Qol-5D (EQ-5D VAS). Handicap and autonomy in ADL was assessed using the London Handicap Scale (LHS)[85] and S&E [6], respectively. Caregivers' burden was assessed with the Zarit Caregiver Burden Inventory (ZCBI)[86] except in institutionalized patients, as a familiar caregiver was absent. At follow-up, patients repeated the same clinical assessment with the exception of the ZCBI and the L-dopa challenge test. Both patients and investigator completed the Clinical Global Impression Improvement Scale (CGI-I) after the L-dopa challenge test and at follow-up.

Assessments were performed at patients' home whenever required by patients' health status or caregiver preference.

Statistical Analysis

Descriptive statistics of demographic, clinical and therapeutic data were provided for continuous [mean and standard deviation (SD)] and categorical (count and percentage) variables.

The acute effect of L-dopa was calculated comparing the MDS-UPDRS-III total score or sub-items, the mAIMS, BP values, VAS-f, VAS-pain, and OH presence/absence in "MED OFF" versus "MED ON", using the t-test, the chi-square test or Fischer's exact test as appropriate. MDS-UPDRS-III sub-items for speech (item 3.1), resting tremor (item 3.17), rigidity (item 3.3), bradykinesia (sum of items: 3.4-3.8 and 3.14), posture (item 3.13), gait

(item 3.10), freezing of gait (item 3.11), arising from chair (item 3.9), and postural instability (item 3.12) were studied separately. Correlations were tested using Pearson's rank correlation coefficient.

For longitudinal analysis, time-course comparisons of paired data sets were performed using Student's t-test (continuous variables) or chi-square (categorical variables) test, as appropriate. Death, being institutionalized in a nursing home or developing HY 5 at one-year follow-up was considered as a combined outcome. Kaplan-Meier survival analysis explored time to the occurrence of death or the combined outcome, whichever occurred first. Differences in the estimated survival distribution stratified by presence of dementia, psychosis, gender, severe dysphagia (MDS.UPDRS item 2.3 >2), and PD phenotype (AK vs. TD) were examined using the log rank test. Statistically significant variables ($p<0.05$) were then used as covariates in Cox-proportional hazard regression model (dependent variable: death alone and combined outcome of death, nursing home or HY 5). If a variable showed border statistical significance ($0.045<p<0.055$), different Cox-proportional regression models were built and the one which minimized the Akaike information criterion was selected. The following variables were entered in the regression model: HY (MED OFF), SE (MED OFF), PDD, MDS-UPDRS-item .2.3 (dysphagia), and NMSS total score.

All p values reported are two-tailed and a $p \leq 0.05$ was considered statistically significant. Coefficients and 95% confidence intervals (CIs) are reported. SPSS 22.0 statistical software (SPSS, Chicago, IL) was used.

Results

Demographic and clinical data at baseline

Fifty LSPD patients were included in the study. Forty patients has a S&E < 50% while thirty-eight patients had a HY > 3 with thirty-two fulfilling both criteria. Forty-six LSPD patients (92%) were observed at home or nursing home due to severe disability. LSPD patients presented a severe clinical picture with a high prevalence of disability milestones (dementia 68%, psychosis 56%, 2 falls per month, wheelchair-bound 18% and nursing home 20%) and NMS (NMSS total score 118 ± 46.6 and NPI-12 total score 21.7 ± 16.2) which negatively affected HR-QoL and caregiver's distress (ZBDS score 28.3 ± 13.3) (Table 1). 38 (76%) of LSPD patients had levodopa-induced motor complications, which were troublesome only in

about a third of the patients (Table 1). Patients with dementia had worse scores of MDS-UPDRS-III, NPI-12 items, NMSS, PDQ-8, LHS and S&E compared to non-demented LSPD patients ($p < 0.05$). PDQ-8 significantly correlated with NMSS and motor impairment ($R = 0.74$ and $R = 0.54$, $p < 0.01$).

Table 1. Demographic and clinical characteristics of LSPD patients

Patients data	**LSPD (n= 50)** **Baseline**	**LSPD (n=36)** **1 year follow-up**	**Baseline vs. 1 year follow-up**
Age (yrs)	77.5 (5.9)	77.8 (7.2)	/
Education (yrs)	6 (5)	/	/
Women (n/total (%))	23/50 (46%)	17/36 (47%)	ns
BMI (Kg/m^2)	22.8 (3.4)	22.3 (3.5)	<0.001
Age at disease onset (yrs)	62 (9.5)	/	/
Disease duration (yrs)	15.5 (6.5)	17 (6)	/
Levodopa treatment duration (yrs)	11.5 (8.9)	/	/
LEDD	1046 (388)	1033 (354)	ns
S&E (ON/OFF)	35.8 (12) / 30 (12)	28.6 (15.1)/NA	<0.001
HY (ON/OFF)	3.8 (0.9) / 4 (1)	3.7 (1.1) /NA	ns
LHS	0.3 (0.11)	0.28 (0.11)	<0.001
HY stage in ON (n (%))	2=8 (16%) 3=5 (10%) 4=24 (48%) 5=13 (26%)	2=6 16%) 3=6 (16%) 4=12 (33%) 5=12 (33%)	ns
Clinical phenotype (n (%)) **Akinetic-Rigid** **Tremor dominant** **Mixed**	 30 (60%) 15 (30%) 5 (10%)	 22 (61%) 12 (33%) 2 (5%)	/
PDD (n (%))	34 (68%)	22 (61%)	ns
MMSE	21.4 (5)	19.7 (7.9)	<0.05
Psychosis (n (%)) Neuroleptic treatment **(n (%))**	28 (56%) 24 (48%)	19 (53%)	<0.001
Falls (n/month) - %	2 (4.4) – 50%	2 (5) – 55%	ns
Gait and walking aid Independent Cane Walker Another person Wheelchair-bound	 5 (10%) 11 (22%) 11 22%) 14 (28%) 9 (18%)	 1 (3%) 10 (28%) 6 (17%) 8 (22%) 11 (30%)	<0.001

Institutionalized	10 (20%)	8 (22%)	<0.05
PEG (n (%))	0	1 (2%)	
Caregiver ^	0 = 27 (54%) 1= 13 (26%) 2= 10 (10%)	0= 21 (58%) 1= 6 (16%) 2= 9 (25%)	ns
ZBDS	28.3 (13.3)	NA	/
Dead (n (%)), **causes**	/	10 (20%) pneumonia (n = 4); not determined (n = 4); intestinal cancer (n = 1); food asphyxiation (n = 1)	/
GDS* **Depression (n (%))** **Light** **Severe**	15.6(4.5) * 34 (68%) 28 (56%) 6 (12%)	14.5 (6.7) 22 (61%) 18 (50%) 4 (11%)	ns ns ns ns
MDS-UPDRS-I, total score Score, mean (SD) - nº of patients scoring positive in the item (%)	**22.2 (7)**	**22.6 (6.7)**	ns/ns**
Cognition	2.9 (1.2) – 92%	3.1 (1.5) – 94%	<0.01
Hallucinations &psychosis	1.4 (1.4) – 54 %	1.3 (1.4) – 50%	ns
Depressed mood	1.9 (0.9) – 88%	2.2 (0.9) – 97%	ns
Anxious mood	1.5 (1.2) – 72%	1.8 (0.9) – 91%	ns
Apathy	1.8 (1.4) - 70%	1.9 (1.4) – 80%	ns
DDS	0.2 (0.5) – 16%	0.2 (1.4) – 2%	ns
Sleep problems	1.4 (1.2) – 68%	1.3 (1.2) – 77%	ns
Daytime sleepiness	1.6 (0.8) – 86%	1.1 (0.8) – 80%	<0.005
Pain	1.6 (1.2) - 74%	1.8 (1.1) – 86%	ns
Urinary problems	2.3 (1.1) – 94%	2.9 (1.1) – 94%	<0.001
Constipation problems	1.7 (1.3)- 74%	1.8 (1.1) – 83%	ns
Light headedness	1.2 (0.9) – 68%	0.7 (0.9) – 44%	<0.05

Fatigue	2.2 (1.2) – 84%	2.1 (0.8) – 91%	ns
MDS-UPDRS-II	**35 (8.9)**	**36.0 (7)**	0.05***
MDS-UPDRS-IV	**4.6 (4.2)**	**3.6 (6.8)**	<0.001****
MDS_UPDRS_III (OFF)	**68.1 (14.1)**	**NA**	/
MDS-UPDRS-III (ON)	**56.4 (15.5)**	**58.5 (14.6)**	<0.005*****
L-dopa induced Motor complications (n (%))	38 (76%)	24 (66%)	<0.01
Motor fluctuations (n (%))	32 (64%)	16 (44%)	<0.01
Troublesome motor fluctuations (n (%))	19 (38%)	11 (30%	<0.01
Dyskinesias (n (%))	23 (46%)	20 (55%)	<0.01
Troublesome Dyskinesias (n (%))	11 (22%)	3 (8%)	<0.01
Painful off-dystonia (n (%))	16 (32%)	11 (30%)	ns
PDQ-8	60.4 (15)	62.1 (17.2)	ns
EQ-5D-VAS	43.7 (14.3) *****	39.7 (15)*	<0.01
NMSS total score	**118 (46.6)**	**128.6 (48.3)**	<0.05
Score, mean (SD) - nº of patients scoring positive in the item (%)			
Cardiovascular	2.7 (3.4) – 61%	1.3 (1.7) - 47%	<0.05
Sleep/Fatigue	12.5 (7.2) – 100%	10 (7.5) – 100%	ns
Mood/Cognition	20.5 (7.2) - 96%	24.2 (18.4) – 97%	ns
Hallucination/perception	6.5 (8.2) – 58%	6.6 (8.6) – 52%	ns
Memory	20 (12.5) – 98%	22.1 (10.7) – 100%	<0.05
Gastrointestinal tract	10 (6.8) – 96%	8.8 (5.2) - 100%	ns
Urinary	17 (11.3) -94%	20.5 (12.9) – 97%	<0.001
Sexual function	20 (6.3) - 100%	23.3 (1.9) – 100%	<0.05
Miscellaneous	9.6 (5.4) 100%	11.5 (6.2)- 100%	<0.01
NPI-12 total score	**21.7 (16.2)**	**23.1 (25.1)**	ns
Score, mean (SD) - nº of patients scoring positive in the item (%)			
Delusion	1.3 (2.2) – 28%	1.5 (2.4) – 42%	<0.001

Hallucinations	2.5 (3.4) -52%	2.8 (3.8) – 50%	ns
Agitation/Aggression	1.9 (3) – 48%	1.5 (1.9) – 50%	ns
Depression	3 (1.9) – 88%	4.7 (3.1) – 97%	<0.001
Anxiety	2.5 (2.5) – 68%	3.4 (2.3) – 88%	ns
Elation/Euphoria	0.1 (0.6) – 6%	0.3 (2.1) – 5%	ns
Apathy/indifference	3.7 (3.7) – 70 %	3.9 (4) – 72%	ns
Disinhibition	0.08 (0.3) – 6%	0.1 (0.7) – 2%	<0.001
Irritability/Lability	1.4 (2.3) – 52%	1.5 (1.9) – 50%	ns
Motor aberrant behaviour	1.7 (3) – 39%	2.2 (3.7) – 38%	ns
Sleep and Nighttime Behavior Disorders	4 (3.3) – 92%	2.4 (3.1) – 92%	ns
Appetite and Eating Disorders	1 (1.5) – 48%	1.1 (1.5)- 50%	ns

Values are presented as mean (SD) if no otherwise specified. HY: Hoehn Yahr Stage; S&E: Schwab and England score; GDS: Geriatric Depression Scale (mild depression: 11- 20; severe depression: 21- 30); LEDD: levodopa equivalent daily dose; PDD: Parkinson's disease with dementia; BMI: Body max index; MMSE: Mini Mental State Examination; EQ-5D VAS: Visual Analogue Scale of the Euro-Qol-5D; PDQ-8: PD questionnaire-8;NPI-12: Neuropsychiatric Inventory test 12-items; ZCBI: Zarit Caregiver Burden Inventory; LHS: London Handicap Scale; NMSS: Non motor symptoms scale; PEG: percutaneous endoscopic gastrostomy; Missing data: (*) → GDS 11/50 (22%) at baseline and 11/36 (30%) at follow-up; ED-5D VAS: 14/50 (28%) at baseline and 2/36 (5%) at follow-up; ^ Caregiver definition: 0= informal at home; 1= formal at home; nurses= 2; 3= not necessary/present; **This significance refers to the progression of MDS-UPDRS – I score of those patients assessed with MDS-UPDRS- I at follow-up (N = 36); the score worsened 0.7 points (±4.0) corresponding to a 8.0% (±24.3) increase. *** This significance refers to the progression of MDS-UPDRS – II score of those patients assessed with MDS-UPDRS- II at follow-up (N = 36); the score worsened 2.3 points (±4.0) corresponding to a 6.0% (±15.0) increase. **** This significance refers to the progression of MDS-UPDRS – IV score of those patients assessed with MDS-UPDRS- IV at follow-up (N = 36); the score improved -1.5 points (±3.8) corresponding to a 20% (±54.8) increase.***** This significance refers to the progression of MDS-UPDRS – III MED ON score of those patients assessed with MDS-UPDRS- III at follow-up (N = 32); the score worsened 7.2 points (±10.0) corresponding to a 15.7% (±23.0) increase. NA: not available; ns: not significant. At MDS-UPDRS-I, NPI 12 item and NMSS a patient was considered as having a "positive" score for the item if score was ≥1; P values for baseline vs. follow-up questionnaires refer to mean values and not to number of affected patients.

LSPD disability progression

Mortality and combined poor outcome. At one-year follow-up (range 12-15 months) 10 (20%) LSPD patients were dead (Table 1). All dead patients were HY 4-5 at baseline. Kaplan Meier survival curves and the log-rank test showed statistical significant difference in the occurrence of the combined poor outcome (death, being institutionalized in a nursing home or developing HY 5) for institutionalized patients at baseline (p = 0.002), patients who needed a formal caregiver (p=0.006) and those with severe dysphagia (MDS-UPDRS item 2.3 >2) (p = 0.001) (Supplementary material: Table S1; and (Figure 1). Institutionalized patients and those with severe dysphagia along with PDD patients had a significant poor outcome even considering only death as final even (p=0.01; 0.003; 0.038, respectively).

Table S1. Log-rank P values for time to "final event" (death/be institutionalized/HY 5)

	Median	**95% CI**	P value
PDD	11,7	10.3 – 13.1	0.25
Non-PDD	13,7	11.4 -16	
Psychosis	11,5	9.8 – 13.1	0.3
Non-psychosis	13,1	11.4 – 14.8	
Male	11	9.2- 12.8	0.1
Female	13,6	12.2 - 15	
Caregiver (formal)	11,2	9.5 – 12.8	**0.006**
Informal caregiver	13,1	11.5 – 14.7	
AK phenotype	11,6	10.1 – 13.1	0.063
TD phenotype	13,9	11.8 – 15	
Institutionalized patients	9,7	8 - 11.3	**0.002**
Non institutionalized patients	12,7	11.2-14	
Moderate/Severe dysphagia	9,3	7.4 – 11.4	**0.001**
No or Mild dysphagia	13,2	11.7 – 14.4	

PDD: Parkinson's disease with dementia; AK: akinetic-rigid; TD: tremor-dominant; Moderate/severe dysphagia (MDS.UPDRS 2.3 item > 2);

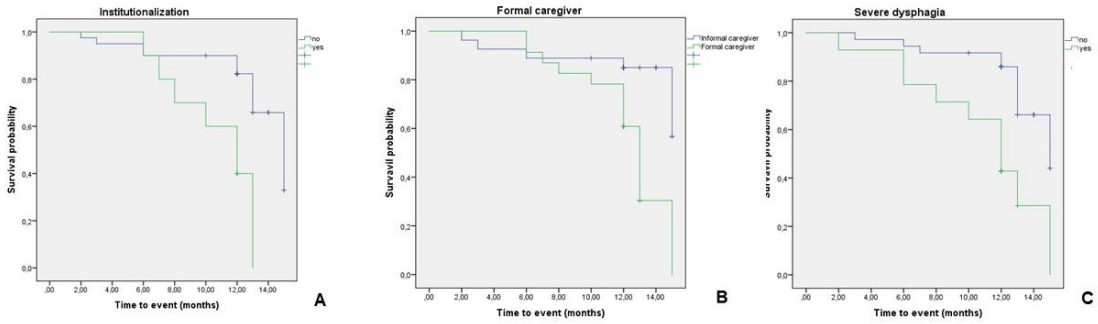


Figure 1. Kaplan-meier curves for the occurrence of the combined poor outcome (death/be institutionalized/HY 5) at follow-up for patients who are institutionalized (A), need a formal caregiver (B) or have a severe dysphagia (C) (MDS.UPDRS item 2.3 ≥2) at baseline;

In multivariate Cox-proportional hazard regression analysis, dysphagia was the only variable that significantly predicted the occurrence of the combined outcome with a hazard ratio of 2.3 (1.1- 4.4, 95% CI; p = 0.01) (Table 2). Dysphagia severity was also the only variable that predicted the occurrence of death with a hazard ratio of 2.9 (1.12- 8.6, 95% CI; p=0.04). Patients with PDD at baseline presented a more significant worsening of dysphagia at follow-up if compared to non-demented patients (p=0.011).

Table 2. Multivariable Cox Proportional Hazards Model for time to death/be institutionalized/HY 5

Variable	**Hazard Ratio (95% CI)**	**P - value**
S&E (MED OFF)	0.97 (0.92 – 1.03)	0.6
HY (MED OFF)	1.2 (0.5- 2.8)	0.3
PDD	0.33(0.16- 3.6)	0.7
NMSS total score	0.55 (0.9- 1.0)	0.5
MDS-UPDRS-item 2.3 (dysphagia)	2.3 (1.12- 4.4)	**0.01**

HY: Hoehn Yahr Stage; S&E: Schwab and England score; PDD: Parkinson's disease with dementia. AK: akinetic-rigid; NMSS: Non-motor symptoms scale.

Motor and non-motor progression. Baseline mean MDS-UPDRS motor score of patients dead at follow-up was significantly worse compared to that of surviving patients, in both ON and OFF state (OFF: 78±12.2 vs 65.5±14.2; ON: 69.6±15.6 vs 53.1±14.6, both p =0.02). Four patients withdrew from the study (3 did not answer to phone calls and follow-up visits could be not scheduled and 1 withdrew informed consent). 36 LSPD patients were examined at one-year visit. During follow-up, 7 patients (14%) were hospitalized and 9 (22%) were institutionalized. Six cases (16%) changed from HY 2-4 to 5, nevertheless median HY stage did not change significantly, though dead patients had a significantly higher HY (OFF and ON) at baseline ($p<0.05$) if compared to survivors. Compared to baseline, there was a statistically significant worsening of motor and non-motor disability, independence in ADL, handicap and HR-QoL. Interestingly, neither the frequency of fallers nor the number of falls\month change significantly at follow-up, but more patients were wheelchair-bound ($p < 0.001$). The mean deterioration of motor score (MDS-UPDRS-III, MED ON) (N = 32) was 7.2 (± 10.0) points corresponding to a 15.7% (±23.0) increase, with no difference between TD vs AK phenotype or patients with/without PDD at baseline. However, 12 patients

(37,5%) had a motor deterioration ≤ 3 points and 14 (43%) ≤ 5 points. Eleven cases (32%) did not deteriorate and, in fact, 10 of these improved between 1-6 points. The mean progression of MDS-UPDRS part II was significantly worse in patients aggravating > 5 points in the motor score compared to those worsening ≤ 5 points or improving in the MDS-UPDRS motor score (2.1±4.1 vs -1.3±2.9, p = 0.01). The score of MDS-UPDRS part IV significantly improved at 1 year follow-up (mean -1.5±3.8 points; 20±50% decrease). Fewer patients had motor fluctuations and troublesome motor fluctuations, although there were significantly more patients with dyskinesias, which nevertheless were less troublesome (Table 1).

The direction of change of NMS between baseline and follow-up differs among scales Table S1). The total score of NMSS worsened significantly while MDS-UPDRS Part I and NPI did not. The frequency of PDD was similar but MMSE score worsened significantly, as did the scores of the items "Cognition" and "Memory" in MDS-UPDRS part I and NMSS, respectively. Despite 5 (13%) developing new psychosis, the number of patients with psychosis significantly decreased at follow-up but the scores of "Hallucinations" item in MDS-UPDRS part I, NMSS and NPI did not change possibly because 8/10 dead patients had a baseline psychosis. The total score of GDS was similar between baseline and follow-up, although the score of "Depression" item in NPI worsened significantly. "Daytime sleepiness" and "Light headedness" (MDS-UPDRS part I) were significantly better at follow-up, as was the "Cardiovascular" domain of NMSS. The scores of "Urinary" significantly increased at follow-up in both MDS-UPDRS-I and NMSS (Table S1).

The score of MDS-UPDRS part II (N = 36) worsened 2.3 points (±4.0) corresponding to a 6.0% (±15.0) increase, and S&E scale also significantly deteriorated between baseline and follow-up. Handicap (LHS) as well as the HR-QoL measured by the EQ-5D-VAS was significantly worse after 1 year, although the change in the PDQ-8 was not significant (Table 1).

Levodopa acute challenge test

The mean MDS-UPDRS-III score was 68.1 (±14.1) in MED OFF and 58.4 (±15.5) in MED ON, with a significant median improvement of 18% (±12) (p<0.001) (Table 3). Sub-analysis of MDS-UPDRS-III scores showed a significant improvement with L-dopa for appendicular

symptoms (rest tremor >> rigidity >> bradykinesia) while no significant changes were noted for axial signs (Table 3).

Measurement of BP in orthostatism was not possible in twelve patients (24%) (two had symptomatic OH, one an amputee leg and nine a severe postural instability). Mean change of SBP from supine to orthostatism as well as mean DBP in orthostatism were statistically different between MED OFF versus MED ON (Table 3). Four patients developed OH in MED ON, which was symptomatic in three (Table 3). 68% of the patients succeeded in completing the VAS scales: pain improved significantly after L-dopa intake, while fatigue did not (Table 3).

We found a significant correlation between the ΔmAIMS and the Δ MDS-UPDRS-III score ($R= 0.64$; $p<0.0.01$). Similarly, MDS-UPDRS-IV total score and dyskinesia/motor fluctuations severity sub-items (4.2 /4.5) had a strong correlation with the Δ MDS-UPDRS-III score ($R= 0.63$ /0.58 respectively; $p<0.001$), whereas, though significant, the correlation was milder for dyskinesia/motor fluctuations duration sub-items (4.1 and 4.3) ($R=0.4$/0.38 respectively; $p<0.05$). No significant correlation was found between Δ MDS-UPDRS-III score and ΔVAS-p. Patients with PDD and AK phenotype had a poorer motor improvement with L-dopa ($p<0.05$). No correlations were found between Δ MDS-UPDRS-III score and PDQ-8, EQ-5D VAS, LHS, S&E and HY. The mean CGI-I scale was 3.1 (±0.9) ("minimally improved") for both patients and investigator, though 12 patients were not able to answer. No serious AEs occurred during the test: eleven cases reported moderate drowsiness or fell asleep after L-dopa, three had symptomatic hypotension and two vomited (Table 3).

Table 3. L-dopa challenge test

LSPD patients (N= 50)			
	MED OFF	**MED ON**	**p - value**
MDS-UPDRS-III	68.1 (14.1)	58.4 (15.5)	**< 0.001**
Speech	2.5 (1.1)	2.5 (1.1)	ns
Rigidity	9.7 (5)	6.5 (5)	**< 0.001**
Bradykinesia	34.5 (6)	31.5 (6)	**< 0.001**
Rest tremor	2,1 (2.8)	0.6 (1.3)	**< 0.001**
Arising from chair	3.3 (0.9)	3 (1)	**< 0.05**
Freezing of gait	2.6 (1.3)	2.4 (1.3)	ns
Postural Stability	3 (0.9)	2.9 (0.9)	ns
Posture	2.3 (0.8)	2.2 (0.8)	ns
Gait	3.2 (0.9)	2.9 (0.9)	**< 0.05**
VAS-p	1.2 (2)*****	0.3 (1.2)*****	**<0.05**
VAS-f	2.8 (3.2)*****	2.8 (3.2)*****	ns
BP_supine	148/80 (31/14)	136/80 (26/17)	**< 0.01**/ns
BP_ortho	142/81 (34/14)	121/75 (30/14)	**< 0.001/< 0.01**
1-OH (n (%))	9 (18%)	13 (26%)	**< 0.05**
2-OH (n (%))	13 (26%)	17 (34%)	ns
AIMS	0.3 (1)	4 (7)	**< 0.001**
S&E	35.8 (12)	30 (12)	**< 0.001**
HY	4 (1)	3.8 (1)	**< 0.01**
L-dopa dose (mg)	336 (102)		
Ocurrence of AEs	11 patients (22%) = drowsiness, 3 patients = symptomatic hypotension (6%), 2 patients (4%) = nausea/vomit		

Values are presented as mean (SD) if no otherwise specified. VAS-p: visual analogue scale for pain; VAS-f: visual analogue scale for fatigue; HY: Hoehn Yahr Stage; S&E: Schwab and England score; BP_supine: blood pressure in clinostatic position: BP_orto: blood pressure after 3 minutes of standing; 1-OT: orthostatic hypotension; 1-OH: defined as decrease in systolic pressure >30 mmHg and in diastolic pressure>15 mmHg, within 3 minutes of standing; 2-OH: defines as decrease in systolic pressure >20 mmHg and in diastolic pressure>10 mmHg, within 3 minutes of standing. **Missing data**: (*) VAS-p and VAS-f 16/50; BP: 12/50;

Discussion

We report the clinical progression of a LSPD cohort over one-year follow-up. After one year, the disease progressed significantly, affecting several motor and non-motor domains and about one-fifth of the cases were dead, institutionalized or changed to HY 5. Severity of dysphagia at baseline is the most important negative prognostic factor for the occurrence of death, institutionalization or HY 5.

As expected, LSPD patients had a high functional dependence, resulting in a severe caregiver distress. Indeed, all need a caregiver and one-fifth lived in nursing home which is possibly influenced by socio-cultural factors or healthcare system organization, although it is similar to that of the UK (14%) and US (25%) [87, 88][17, 18] but lower if compared to the Sydney cohort study at 20 year (48%).[56]

Unexpectedly, we found a high frequency (16%) of HY 2 patients among LSPD group, of whom all but one (with severe axial signs) had PDD with S&E score < 50%. This reflects a previously described limitation of the HY scale, which is heavily weighted toward postural instability[48, 52], and the fact that PD patients may become demented before losing balance. Our data reinforces the usefulness of the S&E scale to identify the whole spectrum of PD patients who entered a late disease stage. LSPD patients had a marked impairment in several NMS domains, with a predominance of urinary, cognitive and sleep disturbances.[54, 76] Frequency of dementia and psychosis is roughly comparable to our previous study,[79] while depression frequency was lower, even though a fifth of the patients were not able to fill the GDS. This frequency rose 20% if taken into account questionnaires filled out with caregivers' help. When comparing our results to the Sydney Multicenter study, we find roughly comparable results for NMS, with a similar prevalence of psychosis (50%), depression (50%), urinary incontinence (about 70%), equivalent values for MMSE score

after 15 years of disease (about 22)[57] and frequency of occasionally chocking (about 50%) with no patient who need artificial feeding in both study, at least at baseline. Over one year, motor and non-motor scores of LSPD patients worsened significantly. Reported annual increase of motor impairment has been estimated around 2.4 points in the UPDRS-III within the first five years of disease[38], with a standardized annual progression rate of 2.4% in intermediate disease stage. [37]Although a slower rate of progression has been reported in more advanced stages of PD, [37] we found a steeper mean deterioration score at the MDS-UPDRS-III, highlighting that a faster disease course could take place in late disease phase. However, this is not homogenous as a considerable percentage of patients deteriorated less than 3 or 5 points, a cut-off that was considered as clinical significant in previous studies, [37] and one-third did not worsen or even improved. This heterogeneity might be due to the death of patients in poorer motor condition during follow-up or medication adjustment after L-dopa test and suggest that only a sub-group of LSPD patients rapidly evolve while stabilization or even improvement of symptoms is still possible. A faster progression of midline motor disability could explain the higher motor score deterioration found in our study.[89] Annual progression rate of 2.2 points in UPDRS-II has been reported [37] for intermediate stage PD patients, which is similar to our findings. Interestingly, L-dopa induced MCs significantly decreased at follow-up despite similar LEDD, confirming the low frequency of troublesome MCs among LSPD.[54, 78]

Among NMS, cognition/mood, urinary and gastrointestinal dysfunction progressed the most. Cardiovascular symptoms seem to decrease. A possible explanation could be the underestimation of these symptoms at follow-up due to cognitive impairment, the fact that BP measurement was not possible in 24% of the cases, the fact that dead patients had a higher thought not significant score for cardiovascular symptoms at baseline or because patients spend more time supine.

Institutionalized patients and those with severe dysphagia have a higher risk of death, institutionalization in nursing home or HY 5 within one year. Nursing home residents with PD may have a 30% higher mortality rate compared to community dwelling patients.[90] In many instances, those patients are under-treated for motor symptoms, although interventions could lead to significant improvements in functioning and QoL.[76, 91] LSPD patients in nursing homes are a fragile subgroup, whose treatment is particularly challenging, as expertise in the management of PD is not uniform among healthcare professionals of nursing homes. In multivariate analysis, only dysphagia predicts a poor outcome. Interestingly,

despite a 28% frequency of severe dysphagia, only one patient had a gastrostomy. Nonetheless, the main death cause was pneumonia and one patient died due to food asphyxiation. As frequent pulmonary infections is the leading cause of death in PD,[92, 93] our results stress the relevance of swallowing monitoring in LSPD patients.

Of note, the magnitude of acute L-dopa response does not predict progression of PD at this disease stage. This may be accounted for a floor effect. In fact, when the magnitude of L-dopa responsiveness decreases below a certain level, its impact on patients' global functioning and disease progression is minimal. In this study, the magnitude of L-dopa responsiveness in LSPD was slightly higher compared to our previous findings (18% vs 11%; 12.7 vs 8.5 points).[78] This difference could be attributed to a larger sample or the inclusion of a larger spectrum of LSPD patients (namely HY 2 cases), even if other clinical features are alike. The clinical significance of this better motor response is marginal according to the CGI-I and the change in the S&E between off and on state. Our results corroborate the unresponsiveness of axial signs to L-dopa in late stage. L-dopa response in LSPD patients was correlated with dyskinesias, adding evidence to our previous suggestion of cautiously increasing L-dopa dose in those patients manifesting MCs or in whom tremor or rigidity are the most troublesome signs. [78] LSPD patients with AK phenotype or PDD had a worse response to L-dopa, which is contrary to previous findings. [89, 94]However, the adoption of different definitions for cognitive impairment and TD phenotype may explain the divergent results. [89]

The strength of our study is to couple data on L-dopa responsiveness with an extensive and longitudinal description of clinical features [55]in a cohort of LSPD patients, who are rarely included in clinical studies. For the first time, we show that dysphagia predicts a worse outcome in these patients and some may still benefit from an increase in L-dopa. [94, 95]

Unblinded clinical assessment is the main limitation of our study. However, our results are in line with ours[78, 79] and others' previous reports,[55-57, 76] giving consistency to our findings.

Conclusion

LSPD is an orphan population expected to increase in the near future and responsible for a high caregiver burden. Their motor and non-motor disability is severe, and 20% is institutionalized in nursing home. Nevertheless, clinical heterogeneity exists and the severity

of axial signs and cognitive decline varies considerably. Consequently, even if disability milestones usually progress exponentially, a slower decline may also be possible. One-fifth dies after one year and the remaining become more disabled. Dysphagia predicts a worse outcome, and attention should thus be taken to a careful assessment and management of swallowing problems. On the other hand, L-dopa responsiveness seems to have no impact on prognosis in this late stage, although L-dopa maintains a slight effect on appendicular signs and especially in those cases with MCs, in whom the dose might be cautiously increased. Nevertheless, higher L-dopa dose will not improve swallowing and non-pharmacological interventions must be prioritized. Future pharmacological and non-pharmacological studies on LSPD patients should be mostly oriented to the management of dysphagia and other L-dopa unresponsive symptoms.

Substantia nigra neuromelanin as an imaging biomarker of disease progression in Parkinson's disease

Abstract

Background: A specific T1-weighted magnetic resonance imaging (MRI) sequence has been shown to detect substantia nigra (SN) neuromelanin (NM) signal changes that accurately discriminate Parkinson's disease (PD) patients from controls, even in early disease stages. However, it is unclear what happens to these SN changes in later disease stages and if they can be a marker of disease progression.

Objective: to investigate the pattern of SN-NM area loss and contrast ratio (CR) intensity changes in late-stage PD (LSPD) compared to earlier disease stages.

Methods: A comparative cross-sectional study was performed, analyzing SN-NM MRI signal in LSPD (Schwab and England Activities of Daily Living Scale score <50 or Hoehn Yahr Stage [HY] >3), comparing this group with *de novo*, 2-5 year PD and controls. SN-NM signal area and CR values for the internal and lateral SN regions were obtained with semi-automated methods.

Results: 13 LSPD, 12 *de novo* patients with PD, 10 PD patients with a 2-5 year disease duration, and 10 controls were included. NM signal area was significantly decreased in LSPD compared to *de novo* PD (*P-value* = 0.005; sensitivity: 75%; specificity 92% and AUC: 0.86). In the lateral SN region, a decrease in the CR was detected in all PD groups compared to controls; despite not reaching statistical significance, a slight increment was observed comparing LSPD to 2-5 year PD. NM signal area significantly correlated with HY (R=-0.37; *P<0.05*) and Movement disorder Society Unified Parkinson's Disease Rating Scale part II (MDS-UPDRS) (R=-0.4; *P <0.05*) while a weak correlation was found with MDS-UPDRS part III (R=-0.26; *P*: 0.1).

Conclusion: SN area evaluated by NM-sensitive MRI may be a promising biomarker of nigral degeneration and disease progression in PD patients.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a selective loss of pigmented neurons in the substantia nigra (SN) *pars compacta* (SNc) and *locus coeruleus* (LC) and by the appearance of Lewy bodies.[96, 97]Approximately 60-70% of dopaminergic neurons of the SNc are lost before the onset of clinical PD symptoms and their degeneration progresses throughout the disease. [98]

The degree of neuronal loss in the SNc is correlated to PD severity, which confirms the potential of SNc imaging for tracking disease progression.[99]

The pronounced depigmentation of SNc neurons is related to the loss of neuromelanin (NM), which, in PD patients, occurs in the whole *pars compacta* region though preferentially affecting the ventrolateral part.[100] Over the last 10 years, new T1-weighted magnetic resonance imaging (MRI) sequences have been shown to detect a significant reduction in the SN-NM signal in PD compared to healthy subjects; these sequences also enable the differential diagnosis with essential tremor. [101] Furthermore, a reduction of SN and LC contrast ratios (CR) has been reported in PD patients distinct from atypical parkinsonian syndromes. [101-105]These NM changes have a high diagnostic sensitivity and specificity for PD diagnosis, even in early clinical stages. [106-109]

However, the relative ability of NM-sensitive MRI to mark disease progression and to detect potential differences in pathophysiological processes still remains unclear. Currently, very few studies have looked at longitudinal changes in the SN NM with MRI; inconsistent results have been reported, that could be related to differences in MR acquisition parameters and data analysis.[106, 108] Likewise, only a few studies have suggested a potential correlation of NM SNpc signal intensity loss (or CR) or NM-volume loss with disease severity, i.e. Hoehn and Yahr rating scale (HY) or Unified Parkinson's Disease Rating Scale (UPDRS) scores.[101, 110, 111]

The purpose of this study was to investigate the pattern of SN-NM area loss and CR intensity changes in late-stage PD (LSPD) patients, compared to *de novo* PD patients and PD patients with a 2-5 year disease duration, and thereby evaluate NM changes throughout disease progression.

Patients and Methods

Patients

We performed a comparative cross-sectional study that included 45 subjects: 13 LSPD, 12 *de novo* PD patients, 10 PD patients with a 2-5 year disease duration, and 10 healthy subjects.

Inclusion criteria for healthy subjects, *de novo* PD patients and patients with a 2-5 year disease duration has already been reported in a previous paper.[106] Patients were recruited from the Movement Disorders Unit of the University Hospital of Santa Maria, Lisbon. PD was defined according to the UK Brain Bank criteria[77] and diagnosis was made by a movement disorders specialist. LSPD was defined as PD patients with either a Schwab and England score (S&E) < 50 (MED ON) or a Hoehn &Yahr stage (HY) >3 (MED ON).[78]

PD patients were rated using the UPDRS, except for the LSPD group who were evaluated by means of the Movement Disorder Society (MDS) UPDRS[49], while MED ON. Conversion from the UPDRS-part II and UPDRS-part III to the MDS-UPDRS part II and MDS-UPDRS part III respectively, was performed adopting the algorithm proposed by Goetz and colleagues.[112] *De novo* PD patients were not on antiparkinsonian medication and they were all <6 months since the beginning of clinical symptoms. L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversions.[81] The Local Ethical Committee approved the study and all patients provided informed consent.

Imaging Protocol

A 3.0 T Phillips scanner (Phillips Achieva; Phillips Medical Systems, Best, Netherlands) was used to acquire all data. A T1-weighted fast spin echo NM-sensitive pulse sequence was used as previously described by Sasaki and colleagues, [113] with a repetition time/effective echo time of 633/10 ms, echo train length of 3, 20 slices with 2.5 mm of thickness and intersection gaps of 0 mm, field of view of 220 mm, matrix size of 548 × 474 (pixel size of 0.40×0.40 mm^2) and an acquisition time of 8 min. Slices were set in an oblique axial plane perpendicular to the fourth ventricle floor and covering from the posterior commissure to the inferior border of the pons. Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) images were also acquired for volumetric analysis, with $0.74 \times 0.74 \times 1.0$ mm^3 resolution, TR/TE of 9.6/4.6 ms. In case of motion artefact, the sequence was repeated adjusting the slice positioning and reiterating to the patient on the importance of remaining still.

Image Analysis

The software OsiriX (OsiriX Lite version 8.0, Pixmeo, Geneva, Switzerland) was used to perform image analysis. A Gaussian filter (full width at half maximum of 0.8 mm) was applied to reduce image noise, prior to performing image segmentation using the confidence region growing algorithm. As the high signal intensity SN was always visible in three slices, the middle slice, corresponding to the greatest SN volume was selected for segmentation.

Two symmetrical seed points were manually defined on the most medial part of the high intensity area in the SN, and as close as possible to an imaginary straight line passing through the bottom of the interpeduncular cistern. The SN CR were assessed by positioning circular regions of interest (ROI), covering approximately 26 pixels, in the internal and lateral parts of both sides of the SN and in the lateral part of the *crus cerebri*, taken as a reference. The CR were calculated using the following equations:

$$CR_{iR} = \frac{SN_{iR}}{CC_R}$$

$$CR_{iL} = \frac{SN_{iL}}{CC_L}$$

$$CR_{lR} = \frac{SN_{lR}}{CC_R}$$

$$CR_{lL} = \frac{SN_{ll}}{CC_L}$$

Where $CR_{iR,iL,lR,iL}$ correspond to the CR of the internal right (iR), internal left (iL), lateral right (lR) and lateral left (lL) regions of the SN, respectively. $SN_{iR,iL,lR,lL}$ are the average values of the signal intensities within the ROIs positioned on the described regions of the SN, and $CC_{R,L}$ the average values of the signal intensities within the ROIs positioned on the right and left region of the *crus cerebri*, respectively (Figure 1).

The midbrain and brainstem volumes were estimated using Freesurfer® for the automatic segmentation of the MPRAGE images. To account for inter-subject variability, the fraction of midbrain to brainstem volume (MBF) was calculated.

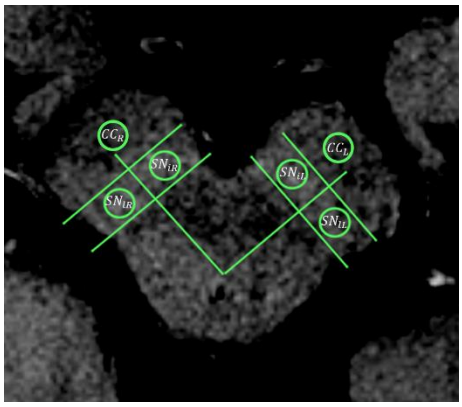


Figure 1. Representative CR assessment by means of circular regions of interest (ROIs) on an NM-sensitive T1-weighted MRI. CC_r: crus cerebri right; CC_L: crus cerebri left; SN_{iL}: substantia nigra, left internal region*;* SN_{lL}: substantia nigra, left lateral region; SN_{iR}: substantia nigra, right internal region; SN_{lR}: substantia nigra, right lateral region.

Statistical Analysis

The Wilcoxon Ranked Test was used to test statistical differences between right and left NM area among subjects of each group. Kruskal-Wallis tests were employed with P-values corrected for multiple comparisons using the Bonferroni method. Potential differences in the SN areas and in the clinical characteristics among the different groups were evaluated. The Wilcoxon signed-ranked test was performed to evaluate differences between the area and CR of both sides of the SN of each subject.

Receiver operating characteristic curve (ROC) analyses were performed to determine the sensitivity, specificity, cut-off optimal values and the area under the curve (AUC) for distinguishing between the different PD groups. The Pearson product-moment correlation coefficient was used to evaluate the dependence between the MDS-UPDRS Part III score, MDS-UPDRS part II, LEDD, HY stage, age and the mean area of the SN and CR*l*/CR*i* results. Also, the dependence between the MBF and the SN areas was evaluated.

Differences in the clinical characteristics were also assessed. The chi-squared test was performed to evaluate differences in the sex distribution among groups. For comparison of the age between groups as well as for the MDS-UPDRS total score and MDS-UPDRS Part III, the Kruskal-Wallis test was used. A P value of 0.05 was considered significant.

All analyses were performed with the R software (Version 3.3.1, The R Foundation for Statistical Computing, Vienna, Austria).

Results

MRI was performed on all subjects, and the image quality allowed a clear identification of the high signal area in the SN region as well as a semi-automatic analysis of all NM-MRI images.

The demographic and clinical characteristics of all subjects are detailed in Table 1. LSPD patients had a median disease duration of 14 years [IQR: 9-17]. They were significantly older compared to controls and *de novo* PD patients and had a worse HY stage and MDS-UPDRS part II compared to the *de novo* and 2-5 year PD groups. MDS-UPDRS part III scores of LSPD patients were worse compared to the *de novo* and 2-5 year PD groups, but the difference was statistically significant only for 2-5 year PD patients (Table 1).

We found no difference between the left and right NM areas ($0.31 < P < 0.79$) and so the mean right/left area value was used in all subsequent analysis.

The median SN-NM area obtained for *de novo* PD patients, 2-5 year PD, LSPD groups and healthy subjects is detailed in Table 1.

	Healthy subjects	***De novo* PD**	**2-5 year PD**	**LSPD**	***P value***
Number (female/male)	10 (4/6)	12 (7/5)	9 (2/7)	13 (7/6)	0.3
Age, yrs	60 [55-69.2]	62.5 [52.5 – 73.7]	66 [63.5 – 71.2]	78 [68.5-81.5]	a, f: 1; b: 0.8; **c: 0.001; d: 0.003**; e: 0.08;
HY	NA	2	2	4	**d - e: <0.001**
LEDD	NA	0	480 [325-810]	1040 [725-1325]	**e <0.01**
MDS-UPDRS part II	NA	6.2 [3.5 – 10.6]	10.1 [1.7 – 12.8]	36 [30-40.5]	**d-e: <0.001**; f: 0.1
MDS-UPDRS part III	NA	32.3 [28.7 – 47]	24.5 [13.4 – 43.1]	51 [41-53.5]	f: 1; **e: 0.02**; d: 0.09;
Area (〖mm〗^2)	40.63 [33.03-55.64]	27.7 [17.13-360.4]	22.65 [8.64- 46.84]	18.68 [12.50 – 26.47]	**a: 0.002; b, c <0.001; d: 0.005;** e: 1; f: 0.8;
CR Internal region	1.16 [1.11 – 1.19]	1.15 [1.09 – 1.21]	1.12 [1.05 – 1.16]	1.12 [1.09 – 1.18]	0.06
CR Lateral region	1.10 [1.02 – 1.12]	1.06 [0.10 – 1.13]	1.03 [0.99 – 1.08]	1.04 [0.10 – 1.1]	**b: 0.008;** a,c:0.1; d, e, f: 1;

Table 1. Demographic, clinical and neuromelanin assessment data of patients and controls. Values are presented as median [IQR: 25th - 75th percentile] if not otherwise specified. NA: not available; LEDD: levodopa equivalent daily dose. CR: contrast ration. HY: Hoehn and Yahr rating scale; MDS-UPDRS: Movement disorders society Unified Parkinson's disease Rating Scale Comparisons: a) controls versus de novo PD; b) controls versus 2-5 yrs PD; c) controls versus LSPD; d) de novo PD versus LSPD; e) 2-5 yrs versus LSPD; f: de novo PD versus 2-5 yrs PD. Statistical significant results are in bold characters.

The median SN-NM area was markedly decreased in PD groups compared to controls (Figure 2) with a *P* value of 0.002 for *de novo* PD patients and a *P* value < 0.001 for 2-5 year PD and LSPD groups (Table 1). The NM area of the LSPD group was significantly smaller when compared with the *de novo* group (*P*=0.005) but not when compared to the 2-5 year PD group (Table 1 and Figure 3).

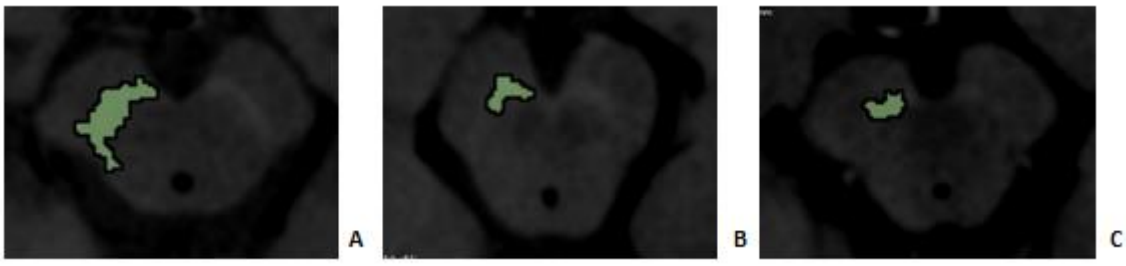


Figure 2. Neuromelanin (NM) are selection on NM sensitive magnetic resonance images of the SN of a healthy control (a), a de novo PD patient (b) and a LSPD patient;

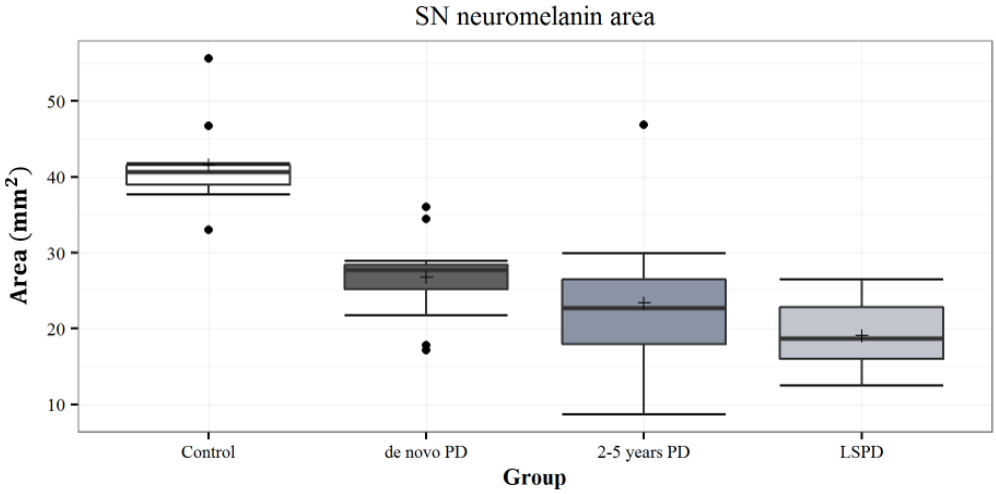


Figure 3. Median area values of the SN high intensity region on NM-sensitive MRI in *de novo PD* patients, 2-5 year PD patients, LSPD patients and controls.

On ROC analyses, the sensitivity and specificity of the SN high signal area for discriminating the LSPD group from earlier PD groups were: a) 75% and 92%, respectively, with a cut-off value for the area set at 26.31 mm^2 and an AUC of 0.86 if compared to *de novo* PD (Figure 3, Panel B); b) 70% and 62%, respectively, with a cut-off value for the area set at 19.29 mm^2 and an AUC of 0.65 if compared to 2-5 year PD; (Figure 4, Panel C). The sensitivity and specificity for discriminating the 2–5 year PD group from the *de novo* group were 67% and 80%,

respectively, with an area cut-off value of 27.16 mm^2 and an AUC of 0.69 (Figure 3, Panel A). Finally the sensitivity and specificity for discriminating all PD patients from controls were 100% and 91%, respectively, with an area cut-off value of 33.02 mm^2 and an AUC of 0.969 (Figure 4, Panel A).

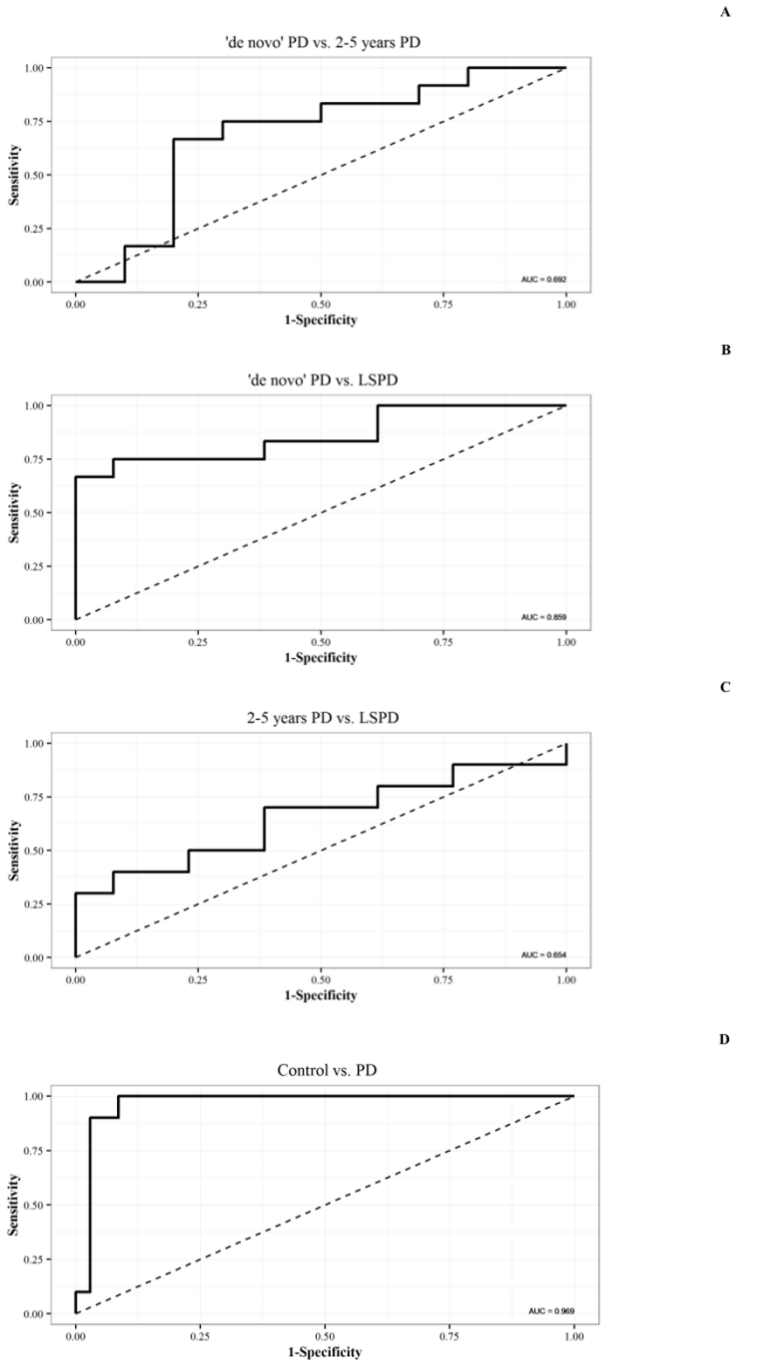


Figure 4. Receiver Operator Characteristics (ROC) curves of the NM area for: a) differentiating between *de novo* PD versus 2-5 year PD patients (A); b) *de novo* PD versus LSPD patients (B); c) 2-5 year PD versus LSPD patients (C); d) PD versus controls.

No differences were found among right versus left CR in both medial and lateral SN across all groups, except for the LSPD group ($P < 0.05$). Thus, CR analysis was performed independently for left and right values. CR analysis for both right and left sides of the internal SN region showed no differences across all PD groups and controls. Concerning the lateral SN region, CR analysis showed a significant difference only for the left side between 2-5 year PD patients and controls ($P<0.05$).

The median left and right CR results obtained for the internal and lateral SN region are detailed in Table 1. Across all groups no differences were found for the internal SN region ($P =0.06$), while CR in the lateral region was significantly different between controls and 2-5 year PD patients ($P =0.008$) (Figure 5). Although no other statistically significant differences were found, a tendency for CR decrease was observed with disease progression for early-intermediate stage groups (Figure 5). Contrary to this trend, an increment in CR was observed for the LSPD group if compared to the 2-5 year PD group (Figure 4).

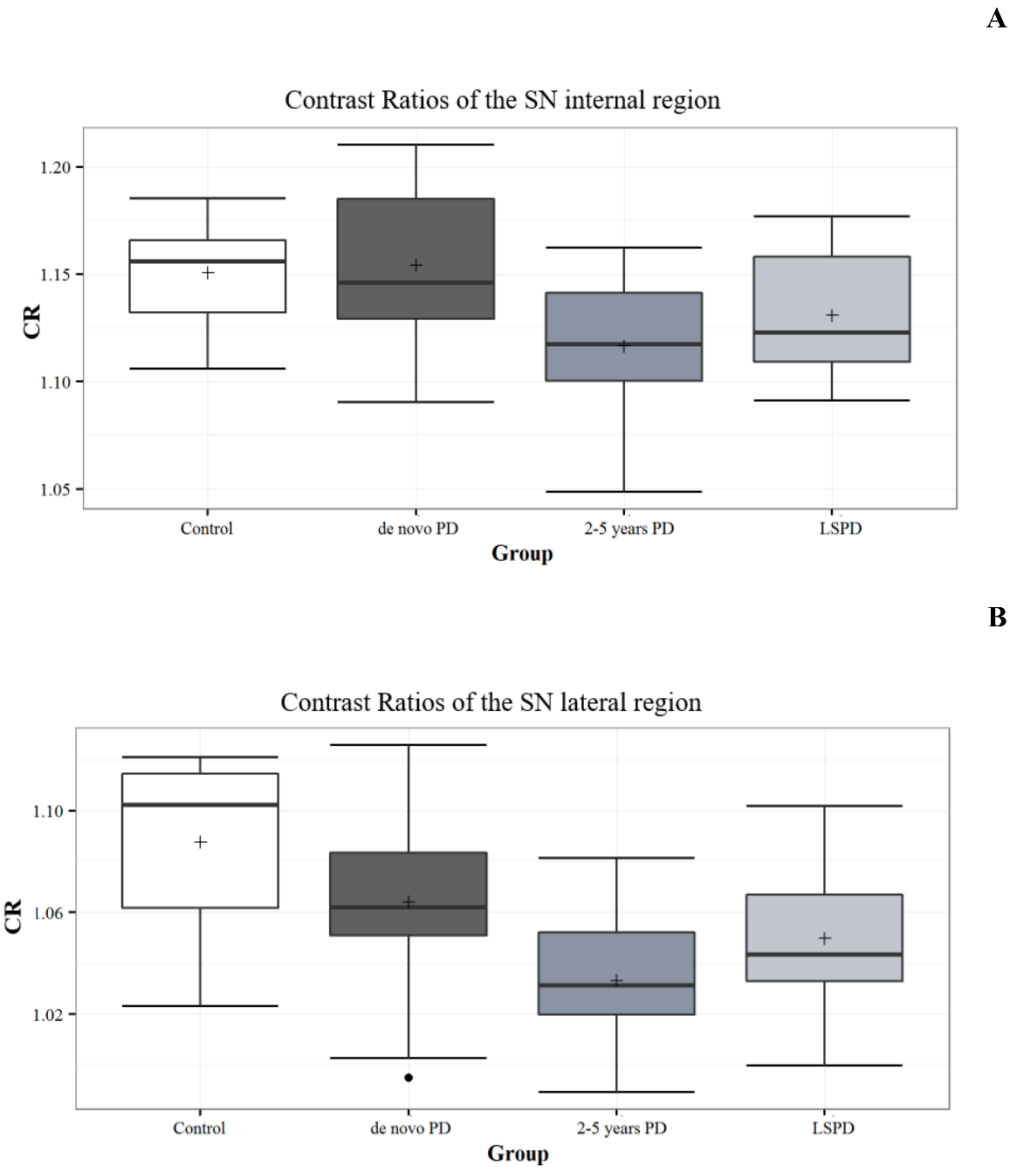


Figure 5. CR values in in *de novo PD* patients, 2-5 year PD patients, LSPD patients and controls for the SN internal region (A) and lateral region (B).

No statistically significant differences were found for the MBF across all groups (global *P*: 0.2) and no correlation was found between MBF and SN-NM area (R= 0.14; *P* = 0.37).

No significant correlation was detected between SN-MN mean area and CR of the internal region (CR*i*) (R=0.33; P=0.054) and the CR of the lateral region (CR*l*) (R=0.3; P=0.08).

Considering all PD groups, MDS-UPDRS part III showed no correlation with SN-NM area (R= - 0.26; *P*: 0.1). Negative moderate correlations were found between the SN-NM area and the MDS-UPDRS part II (R= -0.4; *P <0.05*), LEDD (R= - 0.45; *P <0.05*) and HY (R= -0.37; *P<0.05*). No correlation was found between age and NM area values.

A moderate correlation was found between age and CR*l* (R= - 0.42; P<0.05) and CR*i* (R=-0.36; P<0.05). No correlations were found between HY, MDS-UPDRS part II, MDS-UPDRS part III, LEDD and CR*l* or CR*i*.

Discussion

In the present study, we were able to identify a significant reduction in the NM-SN area compared to controls among several groups of PD patients belonging to different disease stages, i.e. from a very early stage up to LSPD. This is consistent with a tendency for NM depletion with disease progression.

Our results also confirm the ability of NM-MRI related measures for differentiating PD patients from healthy controls with high accuracy, even in the early disease stages, as reported in previous studies. [103, 105-107]

The main objective of our study was to investigate NM-MRI alterations in an LSPD sample, to see the NM changes with disease progression and its potential as a biomarker of disease progression in PD. The NM-SN area presented a tendency to decrease with progressive disease stages, with statistical differences between *de novo* PD and LSPD patients. Furthermore, setting a cut-off value at 26.31 mm^2, we found excellent sensitivity, specificity, and AUC values for differentiating *de novo* PD and LSPD patients (75%, 92% and 0.86, respectively). There are very few studies that have explored NM-area modifications in PD evaluating early, intermediate and advanced PD stages (from HY stage 1 to 4) and all included small sample sizes. These studies reported conflicting results, although the use of different imaging and analysis protocols may partly account for these differences. [108, 110, 114] Indeed, in a previous report we found no differences in SN area or length when comparing *de novo* PD with 2-5 year PD patients.[106] A few other reports suggest a tendency for SN-NM area reduction with disease progression: Schwarz and colleagues observed a tendency for a decrease in NM area when comparing six PD patients with HY stages 1-1.5 with four PD patients with HY stages 2-3.[108]While Aquino and colleagues observed differences in NM area between twenty-two 3-5 year PD and twenty 6-10 year PD patients (HY stage <3).[114] Finally, a recent study by Matsuura and colleagues

reported longitudinal changes in NM-SN area in a group of fourteen PD patients, suggesting a decline of approximately 17.5%, after one year follow-up, concomitant with an aggravation of HY stage (from a range of 1-3 to 2-4).[110] However, to the best of our knowledge, this is the first study in which SN-NM area is specifically examined in a population of LSPD patients. Our findings are in agreement with the report of apparent disease stage- and duration-dependent volume loss of the SN-NM-sensitive region as reported in a manual NM volume analysis, performed on PD patients presenting HY stages 1 to 5.[103] An age-related bias on NM area reduction among our sample of LSPD patients cannot be excluded, as those patients were statistically older when compared to *de novo* PD ones. However, a correlation with age was found only for CR values and not for NM area values. In the current literature there is no consensus on the loss of pigmented neurons during normal aging.[115-117] Nevertheless, throughout a sensitive and specific biochemical quantification of NM, we know that in the SNc this pigment linearly increases with age from the 10th year up to the ninth decade of life. [115, 118]Moreover in normal ageing the fallout of pigmented neurons has a very low rate, i.e. 4.7% per decade.[98] Taken as a whole, our findings on NM area reduction among LSPD patients do not seem to be significantly influenced by age and are more likely accounted for by a stage-dependent modification as opposed to an age-dependent factor.

Though the MDS-UPDRS part III score showed no significant correlation with SN area depletion, we found a negative significant correlation of SN area with other indicators of disease severity, i.e. MDS-UPDRS part II and HY. Such a correlation is in agreement with our finding of NM area stage-dependent depletion, as suggested in a few other studies. [105, 108] The absence of a significant correlation between MDS-UPDRS part III and SN area depletion can be accounted for by the relatively high MDS-UPDRS-III scores of our *de novo* PD sample, probably linked to the medication-free condition of those patients and with the high frequency of tremor dominant type (11 over 12).[101] Moreover, as showed in previous studies, the activities of daily life subscore, i.e. the MDS-UPDRS part II, may be a better biomarker of disease progression than other MDS-UPDRS sections.[119-121]

To evaluate the possible impact of a midbrain volume reduction in PD patients which could have influenced NM measurements, the MBF was calculated for each group. As expected, the midbrain volume was similar between the groups and the calculated MBF showed no correlation with NM area depletion, confirming that individual midbrain volume does not explain the reduction of NM in PD. [106]

Concerning the CR assessment, although a statistically significant difference was observed when comparing PD patients to controls, and a there was a tendency for CR decrease with disease progression, a small and non-statistically significant increment in CR was observed for the LSPD group compared to the 2-5 year PD group. Even if LSPD patients had a clearly worse clinical condition and longer disease duration when compared to 2-5 year PD patients, they were taking a significantly higher levodopa dose. Dopamine and dopamine agonists in standard dosages do not markedly affect DaT binding. A recent study found a correlation of the CR of the SNc and LC with DAT binding values. [122] Interaction between NM-SN signal and dopaminergic therapy is currently unknown but its influence cannot be excluded.

The pattern of pigmented neuron loss of the SN follows an opposite trend comparing PD patients with normal ageing to that observed for CR, with a greatest neuronal loss in PD (45% loss in the first decade), principally affecting the ventro-lateral part of the SN which is relatively spared in controls. [98] Accordingly, comparing healthy subjects with PD patients, we found a significant reduction of CR only in the lateral SN part. Those data suggest that CR*l* could be more appropriate than CR*i* in differentiating PD patients from healthy subjects. A few other studies on NM-CR in PD patients have reported heterogeneous results. Indeed, Ohtsuka and colleagues reported a NM-CR diminishing in the lateral-central part of SNc and LC in early (HY stage 1-2) and advanced (HY stage 3-5, during MED OFF) PD patients, compared to controls, but equally observed no difference between early and advanced patients, which is consistent to results from Schwartz and colleagues[108], however, no LEDDs were reported in either paper. [105] Conversely, Matsuura and colleagues reported a CR reduction during one-year follow-up observation with a correlation between CR values and disease duration, in spite of a LEDD increasing from about 380 mg to 630 mg. [110] Moreover, CR values did not show a significant correlation with indicators of disease severity (HY), further confirming that its alterations are not clearly coupled with disease progression [110] thereby suggesting that other confounding factors should be identified. Myoshi and colleagues found a stage-dependent CR reduction in the medial part of SNc, comparing 1-2 HY PD patients with 3-5 HY ones. [123] Taken as a whole, even if CR of SNc should give a measure of the density of melanized neurons, its relationship with disease progression in PD remains to be clarified. Finally, a greater signal attenuation on NM imaging has been found in the LC when compared to SNc among PD patients[102, 105], though no difference between early and advanced PD patients were found even in the CR of the LC.[102]

A potential source of signal variability is the inhomogeneity in the B1 field, particularly relevant at 3.0T, which is known to affect image contrast. This effect should be accounted for in future studies, performing bias field correction prior to CR evaluation. [124] Future work should include assessing the variability in measured signal intensity and estimated NM-area associated to the acquisition and segmentation procedures. To assess the former, the acquisition procedure should be repeated after patient repositioning.

Several neuroimaging techniques, such as [18F]fluorodopapositron emission tomography (PET), [11C]dihydrotetrabenazinePET, [123I]beta-carbomethoxy-3beta-(4-iodophenyl) tropane single photon emission CT (DAT-SPECT), and [18F]fluorodeoxyglucose PET, have been proposed as markers for nigral abnormalities, disease progression or clinical characteristics for PD.[125, 126] For instance, longitudinal studies have shown an annual rate of reduction in striatal DAT uptake of 6–13% in PD patients.[127, 128] However, these examinations are invasive, expensive, and there is still uncertainty on whether there is an interaction between results and therapeutic intervention outcomes. For this reason, these neuroimaging techniques are not commonly used for routine diagnosis or follow-up of PD patients. Moreover, a very recent study has shown a correlation between striatal DAT density, as measured by DAT-SPECT, and SN-NM volume loss. [122]On the other hand, transcranial ultrasound has also been shown to detect increased echogenicity in the SN in PD as an indirect measure of neuronal loss[129], but this technique is limited by the requirements of a good temporal bone window and its ability in tracking disease progression is still unclear. Recently the loss of the "swallow tail" in the dorsolateral SN as observed at high resolution 3T – SWI MRI has been proposed as an in vivo diagnostic biomarker for nigral degeneration in PD.[109] However even if such a radiological assessment yielded a high diagnostic accuracy (sensitivity 100%, specificity 95%), no longitudinal studies have investigated its modification with disease progression. Our study has several limitations namely the small number of patients in each group and the cross-sectional nature with no longitudinal follow-up. On the other hand, our results clearly show a significant NM signal area reduction in PD patients compared to controls and a tendency for an NM area decrease along with disease progression. These findings are consistent with previous reports and validate the consistency of our results. Due to the small number of patients we were not able to investigate the age-related effect on NM area reduction throughout other statistical techniques (stratification nor regression model). However, no correlation was found between age and area, suggesting a more probable role of disease stage on NM area reduction. NM-MRI has also several technical characteristics that have to be considered when evaluating the

feasibility of performing related imaging studies. It requires a long acquisition time, and the images suffer from relatively low spatial resolution, in-plane signal inhomogeneity and not all image analysis processes are completely automated, although few operator-dependent steps are required. Moreover, motion artifacts during image acquisition and partial volume effects may deteriorate the quantitative nature of the analyses. Nevertheless, we succeeded in performing MRI on all subjects without problems, obtaining good quality images and semi-automated analysis was possible for all patients. Finally there have been, so far, no reproducibility studies of neuromelanin-sensitive MR images. However, there have been up to now several studies using this specific sequence with different equipment and the obtained results are similar in terms of the identification of SN changes in PD patients[108, 114], which is strongly supporting sequence reliability.

Conclusions

In the present study, with semi-automated MRI measures, we detected a stage-dependent progressive decrease in the SN-NM area of PD patients. A marked SN-NM area decrease occurred in parallel with other markers of disease severity. Our findings suggest that NM-sensitive MRI could be used as a potential biomarker for nigral degeneration and disease progression in PD patients. Furthermore, to the best of our knowledge, this is the first study that observed SN-NM area modifications in a sample of LSPD patients, allowing an assessment of the modifications of NM signal in very late disease stage. CR values, although showing a tendency for a decrease with disease progression, presented a slight, albeit not significant, increase in the LSPD group; its interaction with therapeutic intervention and its modifications with disease progression needs further investigation.

Further longitudinal studies on a larger population and the use of consensus acquisition and analysis protocols are warranted in order to replicate our results, verifying if SN-NM area can measure PD patients' progression and if it could be considered as a disease progression imaging biomarker in clinical trials.

CHAPTER 2: Motor response to levodopa in late-stage Parkinson's disease

Do patients with late-stage Parkinson's disease still respond to levodopa?

Abstract

Background: Late-stage Parkinson' disease (PD) is dominated by loss of autonomy due to motor and non-motor symptoms which can be marginally corrected by medications adjustments. However, controversy exists on the mechanisms underlying the apparent decrease of benefit from levodopa.

Objective: To study the response to levodopa in late-stage PD (LSPD).

Methods: 20 LSPD patients (Schwab and England ADL Scale <50 or Hoehn Yahr Stage >3 in MED ON) and 22 PD patients treated with subthalamic deep brain stimulation (DBS) underwent an acute levodopa challenge test. MDS-UPDRS-III and the modified Abnormal Involuntary Movement Scale were evaluated in off and after administration of a supra-maximal levodopa dose

Results: LSPD patients had a median age of 78.8 (IQR: 73.5-82) and median disease duration of 14 years (IQR: 10-19.75). DBS patients had a median age of 66 (IQR: 61-72) and median disease duration of 18 years (IQR: 15-22). LSPD and DBS patients' MDS-UPDRS-III score improved 11.3% and 37% after levodopa, respectively. Rest tremor showed the largest improvement, while axial signs did not improve in LSPD. However, the magnitude of levodopa response significantly correlated with dyskinesias severity in LSPD patients. One third of LSPD and 9% of DBS patients reported moderate drowsiness.

Conclusions: LSPD patients show a slight response to a supra-maximal levodopa dose, which is greater if dyskinesia are present, but it is frequently associated with adverse effects. A decrease in levodopa response is a potential marker of disease progression in LSPD.

Introduction

Patients with Parkinson's disease (PD) develop levodopa-induced motor complications (MCs) after long-term levodopa (L-dopa) treatment. [24]The development of MCs usually defines the beginning of the advanced disease stage. [40] A number of advanced PD patients enter a later stage when motor and non-motor symptoms (NMS) symptoms such as falls and dementia start having a major impact on the health status of patient.[52, 54] In comparison, MCs are less disabling in this late phase. [52]

Recently, we have reported on the clinical characteristics and disabilities of a hospital-based population with late-stage PD (LSPD), highlighting that some of these patients have to decrease dopaminergic therapy due to the occurrence of adverse effects (AEs). [53,54, 130]This raises the question whether the worse motor state of LSPD patients is due to the down-titration of L-dopa because of AEs or decline of levodopa responsiveness due to disease progression.

In order to investigate this, we report here the response of a LSPD population to an acute L-dopa challenge test.

Patients and methods

Objective

To study the motor response of a LSPD population to an acute L-dopa challenge test.

Study design and patients recruitment

This was a cross-sectional study in idiopathic PD patients according to the UKBB criteria.[77]Patients were included in the LSPD group if they had a Schwab and England score (S&E) [60]< 50 or a Hoehn Yahr Stage (HY) >3 in MED ON. The rating of the S&E scale was done by the clinician, interviewing the patient and the caregiver. As an "active control group", we used an advanced stage PD group, defined as patients treated with sub-thalamic nucleus deep brain stimulation (STN-DBS) at least three years before and who did not fulfil the criteria of LSPD. Patients were consecutively recruited from the Movement Disorders outpatient clinic of a tertiary university hospital (Hospital Santa Maria, Lisbon, Portugal). The Local Ethical Committee approved the study and all patients provided informed consent.

Patients assessment

LSPD patients were first assessed at least 12 hours after the last L-dopa/aromatic amino acid decarboxylase inhibitor (LDDCI) intake, 48 hours after the last intake of dopamine agonists, controlled-release LDDCI, selegiline or rasagiline, or 12 hours after the last intake of entacapone (practically defined "MED OFF"/"Condition A"); then, patients were assessed 60-90 minutes after or in the best "MED ON" ("Condition B") condition after a L-dopa intake. For the L-dopa challenge test, each patient took her/his usual morning L-dopa equivalent dose plus 50% (supra-maximal dose=150%). L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversions.[95] Assessments were performed at patients' home whenever required by patients' health status or caregiver preference.

DBS patients were first assessed in the practically defined "MED OFF" condition and with the neurostimulator switched OFF for at least 60 minutes (MED OFF/STIM OFF, "Condition A"). Then, they took the same L-dopa dose as they did in the L-dopa challenge test performed for DBS selection years before (supra-maximal dose), and were assessed again in their best ON (MED ON/STIM OFF, "Condition B").

Motor performance was evaluated using the MDS-UPDRS part III scale[49], the Modified Abnormal Involuntary Movement Scale (mAIMS) and the HY stage during both "Condition A" and "Condition B". Parkinsonism was considered asymmetric when right–left differences in resting tremor, bradykinesia and rigidity were ≥5 points on the MDS-UPDRS items 3.3, 3.4, 3.6, 3.8 and 3.15-3.17. We defined and stratified levodopa-induced MCs according to the following scores: presence of motor fluctuations (MDS-UPDRS 4.3 ≥ 1); troublesome motor fluctuations (MDS-UPDRS 4.4 ≥ 2); presence of dyskinesias (MDS-UPDRS 4.1 ≥ 1) and troublesome dyskinesias (MDS-UPDRS 4.2 ≥ 2). Presence of psychosis was considered if MDS-UPDRS 1.2 score ≥ 1. Clinical phenotypes were defined in both concordant clinical history and the algorithm proposed by Stebbins and coworkers.[131]

Both the patient and the investigator completed the Clinical Global Impression Severity Scale (CGI-S) before the L-dopa test and the Clinical Global Impression Improvement Scale (CGI-I) after the test.

Cognition and mood were assessed during "Condition B" , waiting until any L-dopa related limiting discomfort (e.g. nausea) improved, using the Portuguese version of the Mini Mental State Examination (MMSE), [132]the Geriatric Depression Scale (GDS), and the Pill

Questionnaire. Diagnosis of PD with Dementia (PDD) was made according to the recommendation of the MDS Task Force.[83] Depression was diagnosed if a patient had a GDS score ≥ 11.

Data on demographics, clinical manifestations, disease management, co-morbidities and past medical conditions were obtained using a structured questionnaire (interviewing patients and caregivers), MDS-UPDRS part I, II and IV [49], and review of medical charts when needed.

Statistical Analysis

Descriptive statistics of demographic, clinical and therapeutic data were provided for continuous [median and interquartile range (IQR, 25th–75th percentile)] and categorical (count and percentage) variables.

The acute effect of L-dopa on motor symptoms was calculated comparing the MDS-UPDRS-III score and the mAIMS during "Condition A" versus "Condition B", using the Wilcoxon signed ranked test or the Fischer's exact test, as appropriate. The magnitude of response to levodopa was calculated as MDS-UPDRS-III during MED OFF minus MDS-UPDRS-III during MED ON / MDS-UPDRS-III during MED OFF. The Δ MDS-UPDRS-III was defined as the MDS-UPDRS-III during MED OFF minus MDS-UPDRS-III during MED ON.

MDS-UPDRS-III sub-items for speech (item 3.1), resting tremor (item 3.17), rigidity (item 3.3), bradykinesia (sum of items: 3.4-3.8 and 3.14), posture (item 3.13), gait (item 3.10), freezing of gait (item 3.11), arising from chair (item 3.9), postural instability (item 3.12) and total axial signs (sum of items: 3.1, 3.10-3.12) were studied separately.

Spearman's rank correlation coefficient was used to assess the correlation between the response to L-dopa (Δ MDS-UPDRS-III) with a history and severity of motor fluctuations and\or dyskinesias measured by the MDS-UPDRS IV total score, the MDS-UPDRS items 4.3 plus 4.4 for motor fluctuations and the items 4.1 plus 4.2 for dyskinesias, and with acute onset of L-dopa induced dyskinesias (LIDs), measured by the ΔmAIMS.

Descriptive statistics are reported for the response to L-dopa challenge test for both LSPD and DBS groups. However no direct statistical comparison was done between both groups, as the study was not designed as a case-control study. Indeed LSPD and DBS patients were not matched for any relevant variables (e.g. age, disease duration, duration of levodopa treatment, etc.) refraining the possibility to perform a direct comparison. The advanced stage PD group

was used as an active control group, included to better inform the analysis and interpretation of the results from the LSPD patients.

P value <0.05 was considered significant. SPSS 21.0 statistical software (SPSS, Chicago, IL) was used.

Results

Demographic and clinical data

Forty-two patients were included in the study: 20 LSPD and 22 DBS patients (Demographic and clinical data in Table 1). Seventeen LSPD patients (85%) were observed at home or nursing home due to severe disability. Disability milestones of LSPD patients are detailed in Table 2 while therapeutic data are depicted in Table 3.

Table 1. Demographic and clinical characteristics of LSPD and DBS patients

Patients data	**LSPD (n= 20)**		**DBS (n= 22)**	
Age (yrs)	78.8 [73.5-82]		66 [61-72]	
Education (yrs)	4 [3.25-7]		4 [4-7]	
Women (n/total (%))	11/20 (55%)		12/22 (54%)	
BMI (Kg/m^2)	20.4 [18.5-25.1]		26.1 [24.3-30.2]	
Age at disease onset (yrs)	65.5 [53.5-69.5]		48 [38-54]	
Disease duration (yrs)	14 [10-19.75]		18 [15-22]	
Levodopa treatment duration (yrs)	13 [9.75-20]		16 [12-21]	
Months after DBS	/		57 [44-68]	
Age at DBS (yrs)	/		62 [57-68]	
Asymmetric disease (n (%))	1 (5%)		2 (9%)	
S&E (ON/OFF)	40/30 [30-40/20-30]		90/85 [70-90/67-90]	
HY (ON/OFF)	4/4		2/2	
HY stage in ON (n (%))	2= 1 (5%) 3=2 (10%) 4= 15 (75%) 5= 2 (10%)		1=2 (9%)^ 2=19 (87%) 3=1 (4%)	
Clinical phenotype (n (%)) **Akinetic-Rigid** **Tremor dominant**	Criteria I 11 (55%) 9 (45%)	Criteria II (OFF/ON score) NA /NA	Criteria I 12 (54%) 7 (32%)	Criteria II (OFF/ON score) NA/NA

Mixed **PIGD** **ND**	0 0 NA	9/0 (45%-0%) NA/NA 8/20 (40%-05) 3/0 (15%-0%)	3 (14%) 0 NA	1/0 (4%-0%) NA 20/20 (90%) 1/2 (4%-9%)
PDD (n (%))	14 (70%)		0	
MMSE	20 [16.5-25.5]		29 [27-30]	
Psychosis (n (%))	9 (45%)		4 (18%)	
GDS **Depression (n (%))** **Light** **Severe**	18 [15-19.5]* 14 (82%) 12 (70%) 3 (17%)		13 [6.7-19.5] 13(59%) 6 (27%) 7 (32%)	
CGI-S (investigator)	6 [5-6]*		3 [2.7-4]	
CGI-S (patient)	5 [4-6] *****		3 [3-3.2]	
MDS-UPDRS-I **MDS-UPDRS-II** **MDS-UPDRS-IV** **MDS_UPDRS_III (OFF)** **MDS-UPDRS-III (ON)**	23 [20-27.5] 36 [31.2-40.7] 4 [0.2-7.7] 67 [60.5-78.2] 57 [50.2-64]		14.5 [11.5-24] 18.5 [13.7-23.5] 2.5 [0-8] 52.5 [42-57.5] 19.5 [14-31.2]	
L-dopa induced Motor complications (n(%)) **Motor fluctuations (n (%))** **Troublesome motor fluctuations (n (%))** **Dyskinesias (n (%))** **Troublesome Dyskinesias (n (%))**	15 (75%) 11 (55%) 8 (40%) 9 (45%) 5 (25%)		15 (68%) 10 (45%) 7 (31%) 13 (59%) 9 (40%)	

Values are presented as median [IQR, 25th–75th percentile] if no otherwise specified. GDS: Geriatric Depression Scale ((mild depression: 11- 20; severe depression: 21- 30). BMI: Body max index; MMSE: Mini Mental State Examination. Missing data: (*) → GDS 3/20; CGI-S (patients): 7/20. PIGD: postural instability/gait difficulty. Criteria I: clinical history; Criteria II: Stebbins et al., 2013. ND: not determined; NA: not available. ^: One LSPD patient was HY 2 due to very severe freezing of gait and speech that had a marked impact on ADL.

Table 2. Disability and disease severity milestones of LSPD patients

	LSPD (N=20) Num/total (%)
Gait and walking aid	
Independent	0 (0%)
Cane	3/20 (15%)
Walker	8/20 (40%)
Another person	7/20 (35%)
Weelchair-bound	2/20 (10%)
Falls (last month)	6/20 (30%)
Num/month (median [IQR])	3 [2-5]
Psychosis	9/20 (45%)
Neuroleptic treatment	5/20 (25%)
Neuroleptic treatment without psychosis	2/20 (10%)
PDD	14/20 (70%)
taking rivastigmine \ memantine	7/14 (50%)
Dwelling place	
Home	12/20 (60%)
Home & daytime residential	2/20 (10%)
Nursing home	6/20 (30%)
Time from admission (months) (median [IQR])	48 [IQR: 11-63]
Time to admission (yrs) (median[IQR])	11 [8-26]
CAREGIVER	
Informal (home)	7/20 (35%)
Formal (home)	7/20 (35%)
Formal (Residential care)	6/20 (30%)

Medication	**LSPD (N = 20)**	**DBS (N= 22)**
Levodopa (n (%))		
Total	20 (100%)	16 (72%)
Monotherapy	17 (85%)	5 (22%)
Combination	3 (15%)	13 (65%)
LEED (Median [IQR])	912.5 [760-1160]	555 [312-720]
No anti parkinsonian medication	0	1 (4.5%)
Agonists (n (%))		
Total	0	12 (54%)
Monotherapy		2 (9%)
Amantadine (n (%))	1 (5%)	3 (13%)
Entacapone (n (%))	1 (5%)	1 (4.5%)
Selegiline/Rasagiline (n (%)	1 (5%)	5 (22%)
Neuroleptics (n (%))	5 (25%)	1 (4.5%)
Benzodiazepines (n (%))	8 (40%)	14 (63%)
Antidepressants (n (%))	7 (35%)	13 (59%)
Rivastigmine (n (%))	5 (25%)	0
Quetipiane (n (%))	4 (20%)	1 (4%)
Clozapine (n (%))	1 (5%)	0
Memantine (n (%))	2 (10%)	0
Non-neurological medication (n (%))	15 (75%)	11 (50%)
Stimulation Voltage (median [IQR]) R_STN/L_STN*		3/3 [2.8-3.3]
LEDD before surgery	/	1015 [731-1635]
LEDD after surgery		555 [312-720]

Table 3. Therapeutic data of the patients. LEDD: Levodopa equivalent daily dose. (*): Stimulation frequency was 130 Hz and pulse width was 60 µs for all patients (except for one patient who had a pulse width of 90 µs). All patients were on monopolar stimulation except for one patients who had bipolar stimulation. The median reduction of LEDD was 57% (IQR: 26.5%-65%) after 57 months of DBS.

Levodopa acute challenge test

LSPD patients

The median L-dopa dose for the test was 315 mg [IQR: 277-375]. The median MDS-UPDRS-III score was 67 [IQR: 60.5-78.2] in MED OFF and 57 [IQR: 50.2-64] in MED ON, with a significant median improvement of 11.3% [IQR: 6%-23%] ($p<0.001$) (Table 4). Sub-analysis of MDS-UPDRS-III scores showed a significant median improvement after L-dopa intake for the following sub-items: "rest tremor" 0% [IQR: 0%-93%] ($p<0.05$),"rigidity" 34% [IQR: 7%-87%] ($p<0.001$),"bradykinesia" 11% [IQR: 0%-19%]($p<0.001$). For the 9 patients with rest tremor, the median improvement was 100% [IQR: 12.5%-100%]. Overall Gait had a minimal, but still significant improvement ($p=0.046$); this median benefit was 25% [IQR: 25%-31%] in those four patients showing improvement of gait after L-dopa. No significant improvement was found for all other axial signs (Table 4).

Half of the LSPD patients presented LIDs ($p< 0.005$ for mAIMS), which were generalized in 40% of the cases, involving the lower limbs, neck or trunk in 35%, the face in 30% and the upper limbs in 25% of the cases. The dyskinesias were choreic and mild in 80% of the patients. We found a significant correlation between the ΔmAIMS and the Δ MDS-UPDRS-III score ($R= 0.581$; $p<0.0.5$). Similarly, the MDS-UPDRS-IV total score and the presence of dyskinesias (items 4.1 plus 4.2) showed a significant correlation (respectively: $R= 0.67$; $p<0.05$; and $R=0.634$; $p=0.05$) with the Δ MDS-UPDRS-III score, while no correlation was found with motor fluctuations alone (items 4.3 plus 4.4) ($p=0.8$). A correlation was also found between the MDS-UPDRS-IV and the mAIMS ($R=0.669$; $p<0.05$). Notably, all patients with improved gait after L-dopa (4 patients) had a worse MDS-UPDRS-IV total score and MDS-UPDRS-IV item 4.3 score compared with those who did not have gait improvement ($p=0.05$).

Thirteen patients (65%) succeed in completing the CGI-I scale (median score: 4 - "no change"), while investigators' median score of the CGI-I was 3 ("minimally improved").

No serious AEs occurred during the test: 6 patients (30%) reported moderate drowsiness or fell asleep after levodopa, 5 of them reported sleep problems during the interview (MDS-UPDRS $1.7 \geq 1$).

Advanced stage PD patients

The median L-dopa dose for the test was 350 mg [IQR: 287-450]. The MDS-UPDRS-III total score improved significantly (37% [IQR: 26%-57%]) after L-dopa ($p<0.001$), as did all sub-items with the exception of postural stability (Table 4). Sub-analysis of MDS-UPDRS-III scores showed a statistical significant median improvement of "speech" 0% [IQR: 0%-33%], "rest tremor" 50% [IQR: 0%-100%],"rigidity" 67% [QR: 0%-100%] ($p<0.001$),"bradykinesia" 35% [IQR: 23%-55%], "gait" 25% [IQR: 0%-50%], "freezing" 25% [IQR: 0%-66%], "posture"0% [IQR: 0%-50%], "arising from chair" 0% [IQR: 0%-27%] (Table 4).

No statistically significant difference was found for the mAIMS. Neither the occurrence of LIDs (mAIMS during MED ON\STIM OFF) nor a history of drug-related MCs (MDS-UPDRS- IV), correlated significantly with the response to L-dopa. The median CGI-I score was 2 ("much improved") for both investigator and patients.

Late-stage PD versus advanced stage PD: response to levodopa

Even though no direct statistical comparison has been performed, the magnitude of response to L-dopa in LSPD patients was smaller than in the advanced cohort (Table 4), and this difference was even more marked on axial signs. In spite of a smaller motor response, the occurrence of L-dopa-related AEs was more frequent among LSPD patients.

Table 4: L-dopa challenge test

	LSPD patients (N= 20)				DBS patients (N= 22)			
	MED OFF	MED ON	Effect size (Δ)	p* - value	MED OFF/STIM OFF	MED ON/STIM OFF	Effect size (Δ)	p° -value
MDS-UPDRS-III	67[60-78.2]	57 [50-64]	8.5 [4.7-16.7]	<0.001	52.5 [42.5-58.2]	27 [20-37.5]	18.5 [14-27.5]	<0.001
Speech	3 [2-4]	3 [2-4]	/	1	3 [2-3]	2.5[2-3]	0 [0-1]	<0.05
Rigidity	9 [4-14.25]	3.5 [0-11]	3.5 [1-4.25]	<0.001	4 [1-8.2]	0.5 [0-3]	3 [3-4]	<0.001
Bradykinesia	36,50 [33-40]	33 [24.2-37.5]	4 [0-6.5]	0.001	30 [24.7-32]	19 [11.7-23]	11 [7-16]	<0.001
Rest tremor	0 [0-4]	0	0 [0-2.2]	<0.05	2 [0-3]	0 [0-1]	1 [0-2]	0.001
Arising from chair	4 [3-4]	3.5 [3-4]	/	0.157	0 [0-2]	0 [0-1]	0[0-1]	<0.05
Freezing of gait	3 [2-3]	2 [2-2]	0 [0-0.5]	0.068	1 [0-3]	1 [0-1.2]	1 [0-1]	0.05
Posture	2 [2-3]	2 [2-3]	/	1	1.5 [1-2]	1 [1-2]	0 [0-1]	<0.05
Postural Stability	3 [3-4]	3 [3-3.75]	/	0.059	0 [0-1]	0 [0-0]	/	0.059
Gait	3 [3-4]	3 [3-3.75]	0 [0-0.5]	<0.05	2 [2-3]	2 [1-2]	1 [0-1]	<0.001
Axial Signs	19 [17-22.5]	17 [15-19]	0 [0-2]	0.053	6.5 [5-9]	5 [3-6.2]	2 [1-3]	<0.001
AIMS	0 [0-0]	1.5 [0-9.5]	1.5 [0-8.7]	0.001	0 [0-4]	1.5 [0-6]	0.5 [0-4.5]	0.13
S&E*	30 [20-40]	40 [30-40]	0 [0-10]	<0.05	85 [67-90]	90 [70-90]	0 [0-10]	0.1
HY	4 [4-5]	4	0 [0-1]	<0.05	2	2	/	1
Ocurrence of AEs	6 patiens (30 %) = drowsiness; 1 patients (5%)= symptomatic orthostatic hypotension				2 patients (9%) = drowsiness; 1 patients (4%) = hypertensive crisis			

Values are presented as median [IQR, 25th–75th percentile]. mAIMS: modified Abnormal involuntary movement scale. Statistical significant results are in bold. Axial Signs: sum of item 3.1, 3.10-3.12 of the MDS-UPDRS-III. (*): S&E scores during ON and OFF condition were not evaluated before and after the levodopa challenge test but by means of the clinical interview. p*: MED OFF versus MEN ON; p°: MED OFF/STIM OFF versus MED ON/STIM OFF.

Discussion

As previously reported, [52-54, 56, 57] our new sample of LSPD patients was severely disabled. Now we have found that these patients show a moderate response to a supra-maximal L-dopa dose, although this was frequently associated with the occurrence of AEs.

The response of LSPD patients to L-dopa is poorly understood and it has never been systematically analysed. In a previous study[54], we have identified that a proportion of these patients have difficulties in increasing the dose of dopaminergic therapy, or even had to decrease it, due to AEs. We have now explored whether the motor severity occurring in LSPD is due to the down-titration of dopaminergic drugs, because of AEs, or levodopa-unresponsiveness due to disease progression. Additionally, we applied the same study protocol to a group of advanced stage PD patients that was used as an "active control group". It is acknowledged that DBS patients were selected for surgery because they have a long disease duration, good response to L-dopa and troublesome motor complications, thus they represent a selected group of advanced PD patients. The lack of data on acute L-dopa effect in LSPD patients suggested the evaluation of this group of patients with the same protocol allowing to better inform the interpretation of their results. An earlier PD population not meeting criteria for LSPD, could be also an informative alternative. Moreover we assumed by definition that advanced PD patients were substantially different from LSPD ones, being characterized by a higher L-dopa responsiveness and a lower frequency of dementia and psychosis. However, the choice of an "active control group" was exclusively to inform and validate the results of the study, even though we were aware of the existence of "a priori" clinical differences between the two PD groups.

The motor response of LSPD patients was modest, represented an increase of 11.3 % in MDS-UPDRS-III score. In contrast, a similar L-dopa dose induced a greater improvement (37%) in advanced PD compared to LSPD patients in spite of a higher BMI of the former which is generally associated to a reduced L-dopa's AUC[133], further suggesting that there is a weaker response to an acute L-dopa dose in later stages of PD. However, based on patients' medical charts and clinical history, these LSPD patients had responded well to L-dopa in the past. Rest tremor was the limb symptom that responded best, followed by rigidity and then bradykinesia. Interestingly, this pattern of appendicular symptom response to L-dopa seems to follow that of earlier PD stages.[134] Although gait significantly improved, the median score was 3, in both MED OFF and MED ON, suggesting that this improvement was of no functional relevance.

Similarly, other axial signs did not improve either, thus highlighting the resistance of axial signs to L-dopa therapy compared to earlier PD stages. [117] Axial symptoms classically worsen with disease progression [39, 56, 57] constituting one of the major sources of disability and they mostly become L-dopa unresponsive due to extranigral pathology.[5]

Despite a statistically significant change of MDS-UPDRS-III score, L-dopa had no meaningful clinical implication in the LSPD patients at the CGI-I. Moreover, the change in S&E from 30% in MED OFF to 40% in MED ON, although statistically significant, had very little impact on independence for patients. The lack of benefit perceived by patients is probably due to several factors. First, the acute motor improvement may in fact be minimal and thus not meaningful for patients. Indeed, there is a minimal difference in the motor scores that is judged as clinically meaningful. This minimum clinically important change has been calculated for early PD patients in HY stage 1-3 after 6 months of treatment using the UPDRS and the CGI-I completed by the clinician.[135]Schrag and colleagues determined the minimum change to be a reduction of 5 points in the UPDRS motor score, but no data is available for more advanced stages. [135]Nevertheless, we speculate it would be higher than 5 points for LSPD, and although we found a median reduction of 8.5 points at the MDS-UPDRS-III, it may not be enough to be perceived as meaningful by LSPD patients, as they still had a high MDS-UPDRS motor score in ON.

The second factor potentially affecting the lack of benefit perceived by LSPD patients is their low ability to self-perceive and communicate their opinions due to cognitive decline, speech impairment and the occurrence of drowsiness after L-dopa intake. Finally, patients may conclude that the benefit they get with L-dopa is not strong enough to compensate for the occurrence of troublesome AEs.

We found a positive correlation between L-dopa response and the severity of dyskinesias or the acute onset of LIDs, as previously reported. [58, 136] This suggests that only patients with dyskinesia might gain an additional benefit from L-dopa increment. This probably occurs because dopamine receptors are still sensitive to L-dopa stimulation in these individuals.[137] However, little is known about the pre and post-synaptic functional status of LSPD patients who do not respond to L-dopa at all, particularly whether it is related to striatal cell death. It is likely that the change in motor response to L-dopa in late PD stages is not solely due to pre-synaptic nigrostriatal dopaminergic dysfunction, but also to extra-nigral alterations. Indeed, a loss of striatal dopamine D3 receptors has been correlated with loss of response to dopaminergic drugs and presence of dementia in PD[138]and striatal dopaminergic neurons seem to undergo

structural changes and death with disease progression.[139, 140] Moreover, extra-striatal pathology such as the involvement of the pedunculopontine nucleus in Braak stage 3[1] may underlay postural instability and gait disorder. The absence of severe dyskinesias in LSPD patients during the L-dopa test may be an additional sign of a blunted response to L-dopa.

Notably a third of LSPD patients showed a moderate somnolence during the test while only two DBS patients reported drowsiness in spite of a slightly higher L-dopa dose, suggesting that some L-dopa-related AEs may increase with disease course.

Finally, we have found that LSPD patients have great difficulty in completing several scales, highlighting the hurdles that investigators can face and the lack of proper disease rating scales adjusted to this population disability.

DBS patients had a statistical significant improvement after the acute L-dopa test in all motor sub-items, with the exception of postural stability. This is in accordance with the results of several studies finding a progressive decrement of L-dopa effect in DBS patients with medium/long-term post-surgical follow-up, especially for axial signs.[141, 142] An additional bias that could have enlarged the difference in L-dopa responsiveness between LSPD and advanced PD patients is the younger age at onset for DBS patients. Indeed, it has been shown an increased risk of LIDs in patients with disease onset before the age of 55 and we know that PD patients with earlier motor fluctuations usually present a stronger response to L-dopa and better motor improvement. [135, 136]An interesting finding in our DBS group is the lack of a statistically significant development of dyskinesias after L-dopa intake, supporting the idea that chronic STN high frequency stimulation may induce pharmacodynamics changes and increase the threshold for dyskinesias promoting desensitization to LIDs. [143, 144]

Study limitations

Additional limitations to those addressed above are the small sample size, the unblinded clinical assessments for both patients group's allocation and medication/stimulation conditions, lack of previous data on acute L-dopa effect in LSPD patients and a short washout period for the STIM OFF condition.

We were aware of those limitations during protocol design and accordingly we consider ours an exploratory study that needs future validation. However, to our knowledge, this is the first

study that explores the response to an acute L-dopa challenge test in late phase PD. We cannot exclude a stimulation carry-over effect due to the short washout period of stimulation. Nevertheless, a longer one would probably not be tolerable to patients

Conclusion

In spite of its huge impact on health care systems, LSPD remains an orphan population, barely reached by movement disorder specialists and poorly investigated, but whose prevalence isexpected to increase in the near future. This exploratory study shows that LSPD patients still show a slight response to a supra-maximal L-dopa dose, though this is frequently associated with troublesome AEs. Resting tremor, followed by bradykinesia and rigidity are the main motor features that improve with L-dopa, while axial signs do not change, with the exception of gait in few patients. Even in this late stage, patients manifesting MCs are the ones most responsive to L-dopa.[136] We suggest an increase in the dose of L-dopa in those LSPD patients manifesting MCs in whom tremor or rigidity are the most troublesome motor symptoms. We acknowledge however that an acute benefit with L-dopa may not translate into a long-term improvement and drowsiness may not occur if L-dopa dose is slowly increased. Equally, we are aware on the difference between acute and chronic L-dopa response, warning that stopping completely the L-dopa therapy could slowly and severely aggravate some motor symptoms among LSPD patients.

Our results also suggest that loss of acute responsiveness to L-dopa even in appendicular symptoms might be a sign of disease progression. [136] Finally, the development of better assessment tools that adjust to LSPD patients is a challenge for future clinical research.

CHAPTER 3: Non-motor response to levodopa in late-stage Parkinson's disease

Response of non-motor symptoms to levodopa in late-stage Parkinson's disease: results of a levodopa challenge test

Abstract

Background: Non-motor symptoms (NMS) are extremely common among late-stage Parkinson's disease (LSPD) patients. Levodopa (L-dopa) responsiveness seems to decrease with disease progression but its effect on NMS in LSPD still needs to be investigated.

Objective: To assess the response of blood pressure (BP), pain, fatigue and anxiety to L-dopa in LSPD patients.

Methods: 20 LSPD patients, defined as Schwab and England ADL Scale < 50 or Hoehn Yahr Stage > 3 (MED ON) and 22 PD patients treated with subthalamic deep brain stimulation (advanced PD group) underwent an L-dopa challenge. BP and orthostatic hypotension (OH) assessment, a visual analogue scale (VAS) for pain and fatigue and the Strait Trait Anxiety (STAI) were evaluated before and after the L-dopa challenge.

Results: Systolic BP dropped significantly after L-dopa intake ($p < 0.05$) in LSPD patients, while there was no change in pain, fatigue or anxiety. L-dopa significantly improved ($p < 0.05$) pain and anxiety in the advanced PD group, whereas it had no effect on BP or fatigue. L-dopa-related adverse effects (AEs), namely OH and sleepiness, were more common among LSPD patients. 40% and 65% of LSPD patients were not able to fill out the VAS and the STAI, respectively, while measurement of orthostatic BP was not possible in four LSPD patients.

Conclusions: This exploratory study concludes that some non-motor variables in LSPD do not benefit from the acute action of L-dopa while it can still induce disabling AEs. There is a need for assessment tools of NMS adapted to these disabled LSPD patients.

Introduction

Parkinson's disease (PD) is a multisystem disorder characterized by several motor and non-motor symptoms (NMS).[145] NMS are very common in PD, and their frequency and, in the majority of cases, their severity increase in more advanced stages.[52, 57] Interestingly, the presence, and above all, the severity of levodopa (L-dopa)-induced motor complications (MCs) seem to decrease in late-stage PD (LSPD), [53]thus probably accounting for the major impact that NMS have on patients' quality of life (QoL). Although frequently underdiagnosed[146, 147], NMS play a major role in the QoL of PD patients and carers [6]. Moreover, 30% of PD patients consider L-dopa-induced non-motor fluctuations more disabling than motor fluctuations.[148]

The management of NMS is challenging throughout the disease course, [149]but even more so during the later stages during which patients usually have to decrease dopaminergic therapy due to the occurrence of adverse effects (AEs). [64]Overall, L-dopa responsiveness seems to decrease with disease progression, but very few studies have investigated L-dopa responsiveness among LSPD patients [54, 78], and even less the benefit of L-dopa on NMS. To assess this, we report the response of NMS to an acute L-dopa challenge in a population of LSPD. To better understand the relevance of the results, a group of advanced stage PD patients submitted to sub-thalamic nucleus deep brain stimulation (STN-DBS) underwent the same protocol.

Patients and methods

Objectives

Our primary objective was to assess the response of blood pressure (BP), pain, fatigue and anxiety following an acute L-dopa challenge in an LSPD population.

Design and recruitment

We performed a cross-sectional study in a consecutive sample of LSPD patients, recruited during 6 months from the movement disorders outpatient clinic of a

tertiary university hospital (Hospital Santa Maria, Lisbon, Portugal). PD was defined according to the UK Brain Bank criteria[77], whereas LSPD was defined as PD patients with either a Schwab and England score (S&E) < 50 (MED ON) or a Hoehn & Yahr stage (HY) >3 (MED ON). A group of advanced PD patients was included as an "active control group", to better enlighten the interpretation of both the applicability of the assessment tools and the results. Advanced PD patients were defined as patients treated with STN-DBS at least three years previously, and who did not fulfil the criteria for LSPD. Patients who had undergone DBS were excluded from the LSPD group. The Local Ethical Committee approved the study and all patients provided informed consent.

Assessment of patients

LSPD patients were first assessed in the practically defined "MED OFF" condition and then 60-90 minutes after L-dopa intake in the best "MED ON" condition. Each patient took her/his usual morning L-dopa equivalent dose plus 50% (supra-maximal dose=150%). L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversions.[81]

Advanced patients were first assessed in the practically defined "MED OFF" condition and with the neurostimulator switched OFF for at least 60 minutes (MED OFF/STIM OFF), and then after taking the same L-dopa dose as they did in the L-dopa challenge performed for DBS selection years before (MED ON/STIM OFF). The protocol of the L-dopa challenge performed for DBS selection was the same as for LSPD patients, as previously reported.[78]

NMS were evaluated using the MDS-UPDRS part I[49], the Non-Motor Symptoms Assessment Scale for PD (NMSS)[82], the Neuropsychiatric Inventory test 12-items[150], and the Geriatric Depression Scale (GDS).[151] PD with Dementia (PDD) was diagnosed according to the recommendation of the MDS Task Force.[83]

Depression was diagnosed if patients scored ≥ 11 on the GDS (mild depression between 11 and 20 points; severe depression between 21 and 30 points). Psychosis was present if patients had an MDS-UPDRS item 1.2 score ≥ 1.

Acute response of BP, pain, fatigue and anxiety to L-dopa were assessed immediately before and 60-90 minutes after L-dopa intake in the best "MED ON" condition. BP was measured in supine and 3 minutes after standing; orthostatic hypotension (OH) was defined as a decrease with standing in systolic blood pressure (SBP) >30 mmHg or in diastolic BP (DBP) >15 mmHg (criteria I), or in SBP >20 mmHg or in DBP >10 mmHg (criteria 2). Pain and fatigue were measured using a visual analogue scale (VAS; VAS-p for pain and VAS-f for fatigue). Anxiety was assessed with the State Trait of Anxiety Inventory (STAI), which is a psychological inventory consisting of 40 self-report items, 20 items to assess trait anxiety and 20 for state anxiety, each item is scored on a 4-point Likert-type response scale [18]. For the purpose of our study only the 20 items for state anxiety have been assessed. MDS-UPDRS motor part III[49] was performed in "MED OFF" and then best "MED ON" condition.[78] MDS-UPDRS parts II and IV were used to assess the impact of motor symptoms on activities of daily life and L-dopa-induced MCs, respectively. [49]

Statistical Analysis

Descriptive statistics of demographic, clinical and therapeutic data were provided for continuous [median and interquartile range (IQR, 25th–75th percentile)] and categorical (count and percentage) variables.

The acute effect of L-dopa on NMS was calculated by comparing the median value of BP and the development of OH, and the scores of VAS-p, VAS-f and STAI between MED OFF versus MED ON conditions for LSPD patients and between MED OFF/STIM OFF with MED ON/STIM OFF conditions for DBS patients. Comparisons were made using the Wilcoxon's signed-ranked test or the Fischer's exact test, as appropriate.

Spearman's rank correlation coefficient was used to assess the association between the magnitude of motor (Δ MDS-UPDRS-III) and NMS (ΔVAS-p and Δ VAS-f and Δ STAI) response to L-dopa, and the association between the severity of motor symptoms (MDS-UPDRS-III) and NMS (MDS-UPDRS-I, NMSS NPI-12 items and GDS). Two group comparisons were performed using Fisher's exact test

(categorical variables) and the Mann-Whitney U-test (continuous variables), as appropriate.

LSPD and DBS patients were not matched for relevant variables (e.g., age, disease duration, duration of L-dopa treatment, etc.) thereby not allowing for the possibility of performing direct comparisons between groups, although descriptive statistics are reported. A P value <0.05 was considered significant. The software SPSS 21.0 (SPSS, Chicago, IL) was used.

Results

LSPD patients

Clinical data and NMS characteristics

20 LSPD patients were included in the study. All had had good response to L-dopa in the past. Demographic, clinical, disability milestones, and therapeutic data of these patients have been reported previously [10] and are summarized in Table 1. The application of patients' self-reported scales was hampered due to the presence of dementia and weak cooperation (Tables 1 and 2).

NMS were very frequent and affected all domains (Table 1). PDD was diagnosed in 70% of the patients and hallucinations and psychosis were present in 45% of the cases. Depression was very frequent according to the GDS (88%) and 35% of all cases were taking antidepressants (Table 1).

The overall severity of NMS was moderate-high (MDS-UPDRS part I items scoring ≥ 2 points), namely "cognition", "depressed mood", "anxious mood", "apathy", "day-time sleepiness", "urinary problems", "pain", "light-headedness and fatigue". The NPI-12 documented the presence of "agitation/aggression", "irritability/lability" and "aberrant motor behaviour" in about one-third of the patients. In the NMSS the domains of "mood", "memory", "urinary", "sleep/fatigue", "gastrointestinal" and "sexual" were universally affected (Table 1). The frequency of several NMS was similar across the MDS-UPDRS part I, the NPI-12 and the NMS scales (Table 1).

The caregivers of six patients (30%) reported that their relative frequently spent several hours per day in a sort of apathetic state, with their eyes closed but

apparently not asleep, as they replied if questioned. Among these patients, five (25%) reported the frequent occurrence of a “drowsiness state” 30-40 minutes after L-dopa intake, while anxiety occurring 15-30 minutes before L-dopa intake was reported by two patients.

Table 1. Demographic and clinical characteristics of LSPD and DBS patients

Demographics and clinical features	**LSPD (n= 20)**	**DBS (n= 22)**
Age (yrs)	78.8 [73.5-82]	66 [61-72]
Age at disease onset (yrs)	65.5 [53.5-69.5]	48 [38-54]
Disease duration	14 [10-19.75]	18 [15-22]
S&E (ON/OFF)	40/30 [30-40/20-30]	90/85 [70-90/67-90]
LEDD [IQR, 25th–75th percentile]	912.5 [760-1160]	555 [312-720]
HY (ON/OFF)	4/4	2/2
PDD (n (%))	14 (70%)	0
MMSE	20 [16.5-25.5]	29 [27-30]
MMSE (demented/non-demented)	18 [15-20.5] / 26 [24.7-29.2]	//
Psychosis (n (%))	9 (45%)	4 (18%)
Neuroleptics treatment (n (%))	5 (25%)	1 (4.5%)
GDS Score [IQR, 25th–75th percentile]	18 [15-19.5]*	13 [6.7-19.5]
Depression (n (%))	15 (88%)	13(59%)
Mild	12 (70%)	6 (27%)
Severe	3 (17%)	7 (32%)
Antidepressants treatment (n (%))	7 (35%)	13 (59%)
MDS-UPDRS-I	23 [20-27.5]	14.5 [11.5-24]
Score [IQR, 25th–75th percentile] – nº of patients scoring positive in the item (%)		
Cognition	4 [2-4] – 85%	1 [0-2] – 63%
Hallucinations &psychosis	0 [0-3]- 45%	0 [0-1] – 40%
Depressed mood	2 [1.2-3]- 80%	2 [1-3] – 81%
Anxious mood	2 [0-3]- 80%	2 [0-3] – 68%
Apathy	2 [1-3.7]- 70%	2 [1-2.2] – 86%
DDS	0 – 10%	0 [0-1] – 36%
Sleep problems	1 [0-2]- 65%	1 [0-2] – 63%
Daytime sleepiness	2 [2-2.7] – 90%	1.5 [1-2] – 77%

Pain	2.5 [0-3]- 70%	2 [0-3] -68%
Urinary problems	3 [2.2-3]- 100%	2 [1-2]- 81%
Constipation problems	1.7 [0-2-3.7]- 70%	2 [0-3]- 68%
Light headedness	2 [0.2-2] – 70%	1 [0-1.2]- 59%
Fatigue	3[2-3.7] – 85%	2 [1-3]- 86%
MDS-UPDRS-II	36 [31.2-40.7]	18.5 [13.7-23.5]
MDS-UPDRS-IV	4 [0.2-7.7]	2.5 [0-8]
Painful off-dystonia, Score [IQR, 25th–75th percentile] – nº of patients scoring positive in the item (%)	0 [0-.75] – 20%	0 – 18%
Joint and skeletal deformities (n (%))	4 (20%)	0%
NPI-12 items (total score) *	15 [3-23.5]	8 [2.5-16.5]
Score [IQR, 25th–75th percentile] – nº of patients scoring positive in the item (%)		
Delusion	0 [0-1] – 31%	0 – 0%
Hallucinations	0 [0-1.7] – 37%	0 [0-1] – 27%
Agitation/Aggression	0 [0-1] – 37%	0 – 5%
Depression	1.5 [1-4] – 87%	2.5 [0.7-4.5] – 77%
Anxiety	1 [0.2-4] – 75%	1 [1-4] – 66%
Elation/Euphoria	0 – 0%	0 – 0%
Apathy/indifference	4 [0.2-8.2] – 75%	1 [1-4.5] – 61%
Disinhibition	0 – 0%	0 -0%
Irritability/Lability	0 [0-1] – 31%	0 – 11%
Motor aberrant behaviour	0 [0-1] – 31%	0 – 0%
Sleep and Nighttime Behavior Disorders	2 [2-5.5] – 93%	1 [1-4] – 77%
Appetite and Eating Disorders	2 [2-5.5] – 25%	0 [0-1] – 44%
NMSS (total score)	120.5 [97.7-162.5]	63 [39.5- 77]
Score [IQR, 25th–75th percentile] – nº of patients scoring positive in the item (%)		
Cardiovascular		

Sleep/Fatigue	4 [0-7] - 65%	1 [0-4] - 63%
Mood/Cognition	17 [8.2- 21.5] – 100%	7 [2-12] – 91%
Hallucination/perception	23.5 [8.2-34.2] – 95%	11 [3-19.5] – 95%
Memory	1 [0-12] – 50%	0 [0-2] – 32%
Gastrointestinal tract	27 [6.7-36] – 100%	4 [0.7-7.2] – 77%
Urinary	7 [2.5-19.2] - 95%	5 [3-12] – 95%
Sexual function	13 [9.2-24.7] - 100%	3 [1-7.5] – 81%
Miscellaneous	24 [24-24] – 100%	14.5 [1-7.5] – 95%
	11 [5.7-15.5] – 100%	8.5 [7.5-21.5] – 100%

Values are presented as median [IQR, 25the75th percentile] if no otherwise specified. LEDD: L-dopa equivalent daily dose; GDS: Geriatric Depression Scale. MMSE: Mini Mental State Examination. NMSS: Non-motor symptoms scale; NPI: Neuropsychiatric Inventory Scale; Missing data: (*) /GDS 3/20; the NPI was applied only to 16 LSPD patients and 18 DBS patients.

Levodopa acute challenge test

The median L-dopa dose for the test was 315 mg [IQR: 277-375]. The median MDS-UPDRS part III score was 67 [IQR: 60.5-78.2] in MED OFF and 57 [IQR: 50.2-64] in MED ON, with a significant improvement of 11.3% [IQR: 6%-23%] ($p<0.001$) (Table 2).

Measurement of BP in orthostatism was not possible in four patients (20%) due to their difficulty in remaining in a standing position. Median change of SBP was statistically different between MED OFF versus MED ON ($p < 0.005$). Three and four patients (according to criteria I and II, respectively) developed OH in MED ON, which was symptomatic in only one (Table 2).

Twelve patients (60%) succeeded in completing the VAS scales and 7 (35%) completed the STAI. Pain, fatigue and anxiety did not change significantly after L-dopa intake. There was no correlation between either the ΔVAS-p or ΔVAS-f and the ΔMDS-UPDRS part III while the ΔSTAI correlated with the ΔMDS-UPDRS part III ($R= 0,686$; $p <0.005$). The score of the STAI was not significantly different between fluctuators (score of MDS-UPSRS part IV item $4.3 \geq 1$) and non-fluctuators. Moderate correlation was found between MDS-UPDRS part III (MED ON) and MDS-UPDRS part I ($R=0,675$; $p < 0.05$), GDS ($R=0,634$; $p < 0.005$) and NMSS ($R=0,695$; $p< 0.05$), but

not with NPI-12 items, indicating that a worse motor condition was associated with more severe NMS. Severity of motor parkinsonism was not significantly different between demented and non-demented patients, whereas PDD patients had worse scores of MDS-UPDRS parts I and II compared to non-demented patients.

No serious AEs occurred during the test. Six patients (30%) reported moderate drowsiness or fell asleep after L-dopa. The occurrence of L-dopa-related AEs was neither associated with longer disease duration, older age, age at PD onset, PDD, L-dopa dose, nor with a worse motor score (MED ON).

Table 2. NMS response to L-dopa

LSPD patients (N = 20)					DBS patients (N = 22)			
	MED OFF	MED ON	Effect size (Δ)	p* - value	MED OFF/STIM OFF	MED ON/STIM OFF	Effect size (Δ)	p° -value
MDS-UPDRS-III	67.5 [60.6-78.2]	57 [49-64]	8.5 [4.5-16.7]	**<0.001**	52.5 [42.5-58.2]	27 [20-37.5]	18.5 [14-27.5]	**<0.001**
STAI	47.5 [41.2-52.7]^	41 [30-49]^	4 [0-22]^	0.1	50.5 [43.7-59.2]	37.5 [33-45]	13 [9-19.2]	**<0.001**
VAS-p	0 [0-4.5]*	0 [0-3]*	/	0.07	0 [0-5]	0	0 [0-3.5]	**<0.05**
VAS-f	5 [0-8]*	5 [0-5.7]*	/	0.2	2.5 [0-7]	1.5 [0-4.2]	0 [-2.5-5]	0.2
BP_supine	157/83 [135/83-174-90]	134/80 [111/78-170/95]	23 [1-38] /2.5 [-11-9]	**0.004**/ 0.7	147/90 [136/79-170/98]	145/90 [130/79-172/98]	/	1/0.133
BP_ortho	147/85 [127/69-178/93]°	105/75 [90/63-140/90]°	26 [0-49]/ 7 [-11-10]	**0.002**/ 0.2	147/93 [125/85-177/100]	139/89 [119/76-153/98]	12/5 [-9/24- -5/20]	0.1
1-OH (n (%))	4 (20%)°	7 (35%)°	3 (15%)	0.1	3 (13%)	5 (22%)	4 (18%)	0.5
2-OH (n (%))	4(20%)°	8 (40%)°	4 (20%)	**<0.05**	4 (18%)	7 (31%)	5 (22%)	0.3
	30 [20-40]	40 [30-40]	0 [0-10]	**<0.05**	85 [67-90]	90 [70-90]	**0** [0-10]	0.1

S&E								
HY	4 [4-5]	4	0 [0-1]	**<0.05**	2	2	/	1
Ocurrence of AEs	6 patiens (30 %) = drowsiness; 1 patients (5%)= symptomatic OH				2 patients (9%) = drowsiness; 1 patients (4%) = hypertensive crisis			

Values are presented as median [IQR, 25the75th percentile]. STAI: State Trait of Anxiety Inventory (only the 20 items of state anxiety have been applied); VAS-p: visual analogue scale for pain; VAS-f: visual analogue scale for fatigue; BP_supine: blood pressure in supine position: BP_orto: blood pressure after 3 min of standing; OT: orthostatic hypotension HY: Hoehn Yahr; S&E: Schwab and England score; p*: MED OFF versus MEN ON; p_: MED OFF/STIM OFF versus MED ON/STIM OFF. Missing values/STAI: ^13 over 20; VAS: * 8 over 20; BP: _ 4 over 20; 1-OH: defined as decrease in systolic pressure >30 mmHg and in diastolic pressure>15 mmHg, within 3 min of standing; 2-OH: defines as decrease in systolic pressure >20 mmHg and in diastolic pressure>10 mmHg, within 3 min of standing.

Advanced PD patients

Clinical data and NMS characteristics

22 DBS patients were included in the study and, overall, NMS were less severe in advanced patients compared to LSPD (Table 1). No advanced patient was demented, 18% reported hallucinations and depression was diagnosed in 59% of patients. The following items scored ≥ 2 points in the MDS-UPDRS part I, indicating moderate-high severity: "depressed mood", "anxious mood", "apathy", "pain", "urinary problems", "constipation" and "fatigue". Interestingly, joint and skeletal deformities were absent.

Levodopa acute challenge test

The median L-dopa dose for the test was 350 mg [IQR: 287-450]. The MDS-UPDRS-III score improved significantly (52.5 versus 27; 37% [IQR: 26%-57% $p < 0.001$]) after L-dopa (Table 2).

The intake of L-dopa had no significant effect on mean BP and fatigue. Four and five patients (according to criteria I and II, respectively) developed asymptomatic OH in MED ON (Table 2). L-dopa improved pain and anxiety ($p < 0.05$). The ΔVAS-p did not correlate with ΔMDS-UPDRS-III. On the other hand, the ΔSTAI had a moderate correlation with the magnitude of L-dopa response ($R = 0,427$; $p < 0.05$) but not with presence of "wearing-off" or "dyskinesias" (MDS-UPDRS-IV item 4.3 and 4.1). A moderate correlation was found between MDS-UPDRS part III (MED ON/STIM OFF) and the NMSS ($R=0,427$; $p<0.05$) but no correlation was found with the MDS-UPDRS part I or the NPI-12.

Discussion

As previously reported, we found a high frequency and severity of NMS among LSPD patients, [56, 57, 147] which were correlated with motor disability. All domains of NMS were involved and most domains affected all patients. Frequency of NMS was similar among different scales, giving internal consistency to our results. We were able to perform an L-dopa challenge on these very disabled patients, although the difficulty encountered by patients completing the self-reported scales possibly

hampered the assessment of the response of NMS. Despite this, the results showed no significant effect of an acute L-dopa challenge on pain, fatigue or anxiety, while SBP decreased after L-dopa intake and OH emerged in about 25% of tested patients. Additionally, AEs occurred in one-third of patients after the intake of L-dopa, namely sleepiness. Furthermore, we applied the same study protocol to a representative group of advanced stage PD patients who were used as an "active control group". The lack of data on acute L-dopa effect on NMS in LSPD patients suggested the need to assess this group of advanced PD patients in order to validate the assessment tools and enrich the results.

We decided to restrict the assessment of NMS only to some symptoms, namely pain, fatigue, anxiety and BP, the specific acute modifications of which could be evaluated during an L-dopa challenge in an in a frail population of LSPD population with a high frequency of dementia and speech difficulties and using relatively simple tools. Indeed, the majority of instruments available to assess NMS in PD may be inadequate in very disabled patients, similarly to other neurodegenerative conditions.[152] Such burden is a specific trait of LSPD patients, as we found no similar difficulties for the group of advanced PD patients. There is the additional risk of low reliability of LSPD patients' response to self-reported scales or questionnaires due to cognitive and speech impairments and the occurrence of AEs after L-dopa.

Nevertheless, we diagnosed probable dementia in 70% of LSPD patients, which is quite high compared to other case series (45%-50%) with similar disease duration, [54, 57]while the frequency of psychosis was similar to previous reports (about 45%).[54, 57] Depression was diagnosed in 88% of patients and the difficulty encountered in completing the GDS may have nevertheless resulted in an underestimation of its frequency and severity. The frequency of mild depression (70%) was found to be rather high, but almost half of the depressed patients were not taking antidepressants, which highlights how depressive symptoms may go unnoticed in such a late phase, or, alternatively, that antidepressants were discontinued in the past due to AEs. Dysautonomic symptoms were equally very frequent and bothersome to LSPD patients. The high frequency of daytime sleepiness, apathy and motor aberrant behaviour in LSPD patients results in a severe

clinical picture, in which patients spend most part of the day alternating between an “apathetic state” with eyes closed and periods of excessive sleepiness or purposeless motor behaviour.

The acute L-dopa challenge induced a 23-mmHg drop in SBP and the occurrence of OH in one-fourth of patients. OH was symptomatic only in one patient, which contrasts with the high frequency of symptoms of orthostatism. Diagnosing and treating low BP in LSPD may prove beneficial in improving patients’ handicap. Interestingly, L-dopa did not cause a significant decrease in BP in advanced PD patients, who had longer disease duration, suggesting that the severity of dysautonomia may not be determined solely by disease duration.

The intake of L-dopa did not significantly change the severity or the frequency of pain, fatigue and anxiety. This contrasts with the significant improvement of both anxiety and pain among advanced PD patients, possibly linked in part to their better motor response to L-dopa. Alternatively, the major source of pain in LSPD patients may be related to secondary causes such as radicular compression, musculoskeletal deformities and contractures, which do not respond to L-dopa and the treatment of which is challenging.[64] In fact, the frequency of painful off-dystonia, highly responsive to L-dopa, was similar for LSPD and DBS patients, but two-thirds of patients reported some discomfort due to pain, suggesting that other causes of pain could have a greater impact on patients.[153, 154]

The absent effect of L-dopa on fatigue in both populations is not surprising. Indeed, even if L-dopa has been proposed to induce a slower progression of fatigue compared with placebo,[155] currently no treatment is considered effective for this NMS,[156] and dopaminergic pathways seem to be only partially involved in the pathogenesis of fatigue in PD. [155] Even though the same seems true for anxiety, the rate of missing data among LSPD patients is too high to draw any firm conclusion. In fact, severity of anxiety moderately correlated with the motor improvement with L-dopa in both groups of patients. The acute effect of L-dopa on anxiety has been investigated in a few studies with small and heterogeneous samples of non-demented PD patients in intermediate/advanced stages. The findings suggest that L-dopa improves anxiety

that fluctuates with L-dopa intake, whose magnitude is stronger in patients with motor "wearing-off" and that the fluctuation of anxiety correlates with the magnitude of motor response.[157, 158] Accordingly, anxiety significantly improved after L-dopa in our advanced patients whose motor response to L-dopa was greater than in the LSPD group. The absent effect of L-dopa on anxiety among LSPD patients could be additionally explained due to a wider neurodegeneration of the locus coeruleus in the latest disease phase, which has been implicated in the pathogenesis of anxiety in PD.[1, 159] Moreover, the lack of effect of L-dopa on anxiety in LSPD patients could also be related to the presence of an Alzheimer's disease-type pathology among LSPD patients, in which the presence of depression and anxiety may be mainly related to the presence of dementia.[160, 161] Despite a lower L-dopa dose, the frequency of L-dopa-related AEs is slightly higher among LSPD patients than advanced ones. We may speculate that these AEs increase progressively with disease progression and the presence of dementia. Nevertheless, we did not find any correlation between frequency of AEs and disease duration, age, age at PD onset, PDD or disease severity of LSPD patients. The presence of these AEs, such as symptomatic OH, daytime sleepiness or hallucinations, frequently implies L-dopa dose reduction, making it even more difficult to manage PD in this late stage.

It could be interesting to investigate the acute and long-term effect of levodopa-carbidopa intestinal gel (LCIG) on NMS among LSPD patients. Indeed, some recent reports suggest an improvement of some NMS such as sleep/fatigue, pain, gastrointestinal and urinary symptoms, as assessed by the NMSS, during chronic treatment with LCIG.[162-164] Nevertheless the level of evidence for improvement of NMS is still considered low [165]and no study has specifically addressed LSPD patients.

Study limitations

The sample size of the LSPD group was small, although these patients are very difficult to recruit.[54] The washout period for the STIM OFF condition in the advanced group was short, but many patients could not tolerate longer time without stimulation.

On the other hand, we could have investigated more NMS and also the several causes of pain in PD[153] and how they might respond differently to an L-dopa acute challenge. Importantly, our results concern the response of NMS to an acute intake of L-dopa and thus it may not indicate how these NMS respond to a chronic intake of L-dopa.

Conclusions

To the best of our knowledge, this is the first study that explores the response of non-motor variables to an acute L-dopa challenge in LSPD. Our exploratory study confirms the high severity and frequency of NMS among LSPD patients, and highlights the need for assessment tools adapted to these very disabled PD patients. Some NMS such as pain, fatigue and anxiety do not benefit from the acute action of a supra-threshold dose of L-dopa, which is in line with our recent findings for motor symptoms [78]and suggests an overall decrease of the effect of L-dopa with disease progression, at least its acute effect. Despite this, L-dopa retains the ability to induce AEs in LSPD patients; these AEs may possibly not occur if L-dopa dose is slowly increased. We acknowledge, however, that the benefit from an acute L-dopa challenge for pain, fatigue and anxiety in earlier stages of PD is not well established, in contrast to the amount of evidence of its effect on motor symptoms. Thus, we can speculate that clinicians should not expect any gain from L-dopa dose increase for those NMS in LSPD patients. In fact, they should be cautious when trying to increase the dose of L-dopa, as frequent L-dopa-related AEs may occur, namely somnolence and arterial hypotension. They should indeed try to decrease L-dopa dose when facing troublesome daytime somnolence or arterial hypotension.

The expected increase in the prevalence of this orphan population, the limitation of current assessment scales and the apparent lack of response of certain NMS to L-dopa highlight the need for larger studies of LSPD in order to optimize the

assessment of these patients and the treatment of NMS, which are a major source of disability in later PD stages.

CHAPTER 4: Speech response to levodopa in late-stage Parkinson's disease

Speech and voice response to a levodopa challenge in late-stage Parkinson's disease

Abstract

Background: Parkinson's disease **(**PD) patients are affected by hypokinetic dysarthria, characterized by hypophonia and dysprosody, which worsens with disease progression. Levodopa's (L-dopa) effect on quality of speech is inconclusive; no data are currently available for late-stage PD (LSPD).

Objective: To assess the modifications of speech and voice in LSPD following an acute L-dopa challenge.

Method: LSPD patients (Schwab and England <50/Hoehn Yahr >3 [MED ON]) performed several vocal tasks before and after an acute L-dopa challenge. The following was assessed: respiratory support for speech, voice quality, stability and variability, speech rate and motor performance (MDS-UPDRS-III). All voice samples were recorded and analyzed by a speech and language therapist blinded to patients' therapeutic condition using *Praat* 5.1 software.

Results: 24/27 (14 men) LSPD patients succeeded in performing voice tasks. Median age and disease duration of patients was 79 [IQR: 71.5-81.7] and 14.5 [IQR: 11-15.7] years, respectively. In MED OFF, respiratory breath support and pitch break time of LSPD patients were worse than the normative values of non-parkinsonian. A correlation was found between disease duration and voice quality (R=0.51; p=0.013) and speech rate (R= -0.55; p=0.008). L-dopa significantly improved MDS-UPDRS-III score (20%), with no effect on speech as assessed by clinical rating scales and automated analysis.

Conclusion: Speech is severely affected in LSPD. Although L-dopa had some effect on motor performance, including axial signs, speech and voice did not improve. The applicability and efficacy of non-pharmacological treatment for speech impairment should be considered for speech disorder management in PD.

Introduction

Parkinson's disease (PD) patients are classically affected by hypokinetic dysarthria, characterized by hypophonia and dysprosody that worsens with disease progression due to breathing, phonation, and articulation dysfunction. [166-168]Speech disorders affect nearly 90% of PD patients and have a negative impact on functional communication, which in turn contributes to decreased quality of life.[169, 170] Symptoms vary from a soft and breathy voice that lacks modulation in volume (monoloudness) and fundamental frequency (monopitch or monotone) resulting in flat speech melody (dysprosody), with pitch breaks, lack of rhythm and pace of speech, number of pauses, reduced stress and imprecision in consonant articulation, to a voice that is neither audible nor intelligible.[171-174]

The effect of levodopa (L-dopa) on the quality of speech is inconclusive given that it is also influenced by each patient's speech profile. Some studies report on a slight improvement of intonation, vowel articulation, and speech intelligibility[175-178], while others show no significant effect[179, 180] as measured during an acute L-dopa challenge. Nevertheless, speech is generally considered to be a "L-dopa-resistant" axial motor symptom of PD.[181] Axial impairment is preponderant among PD patients in the latest disease stage,[52] although no data are currently available on the effect of L-dopa on speech among late-stage PD (LSPD) patients. The purpose of this study was to assess the clinical and active modifications of speech and voice after an acute L-dopa challenge in a LSPD population.

Patients and methods

Design and recruitment

We performed a cross-sectional study in a consecutive sample of LSPD patients recruited during 12 months from the movement disorders outpatient clinic of a tertiary university hospital (Hospital Santa Maria, Lisbon, Portugal). PD was defined according to the UK Brain Bank criteria,[77] whereas LSPD was defined as PD patients with either a Schwab and England score (S&E) < 50 (MED ON) or a Hoehn & Yahr stage (HY) >3 (MED ON).[78] The Local Ethics Committee approved the study. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Assessment of patients

LSPD patients were first assessed in the practically defined "MED OFF" condition and then 60-90 minutes after L-dopa intake in the best "MED ON" condition. For the L-dopa challenge each patient took her/his usual morning L-dopa equivalent dose plus 50% (supra-maximal dose=150%). L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversions.[81] Details of the L-dopa challenge have been previously reported. [78]

The following parameters were assessed during both MED OFF and MED ON: a) motor performance by means of the MDS-UPDRS part III[49]; b) severity of dyskinesias using the Modified Abnormal Involuntary Movement Scale (mAIMS); c) respiratory support for speech (time duration of vowel /a/ prolongation); d) voice quality (fundamental frequency [F_0]); e) voice stability (pitch break time and jitter); f) voice variability (standard deviation [SD] of speaking F_0 during sentences [Sentence F_0SD]); g) speech rate (syllables/sec). Each participant had to perform several vocal tasks that consisted of: (i) sustained phonation of the vowel /a/ at a comfortable pitch and loudness and (ii) repeating an 8-word, 14-syllable standard statement/declarative sentence, 'A Maria comprou-me um mapa do papel branco.' [translation: Mary bought me a map of white paper]; (iii) reading five words and five sentences. Tasks were selected from the European Portuguese version of the Frenchay Dysarthria Assessment version 2.[182] However, due to the low level of cooperation of LSPD patients, we adopted an 8-word (14 syllables) declarative sentence (syntactically simple) that in European Portuguese is expected to have a low level of voice variability compared to complex sentences or text reading, which are normally used for this task.

Patients were seated and instructed by a neurologist to sustain the vowel /a/ at a comfortable pitch and loudness as long as they could. A demonstration was made by the clinician before the patient performed each vocal task. There were no time limits for each participant and he/she was asked to repeat the task if the examiner was not fully satisfied with patient's performance. All voice samples were recorded in a room in a home environment using a tabletop unidirectional microphone (Fame, MS-1800S) attached to a preamplifier (M-Audio Fast Track Pro, preamp, USB) and a desktop computer running *Audacity software version 2.1.2* (Free software Foundation Europe, Hamburg, Germany).

Two separate perceptual files were completed using *Audacity software* version 2.1.2 with all the stimuli presented at the same sound pressure levels and with a 500 ms silence between single words and sentences.

MDS-UPDRS parts II and IV were used to assess the impact of motor symptoms on activities of daily life and L-dopa-induced motor complications, respectively. PD with Dementia (PDD) was diagnosed according MDS Task Force recommendations.[83]

2.3 Data analysis

All voice samples were copied to a computer (down sampled to 24kHz, 16 bits, mono), edited into individual files and screened for extraneous noise using *Audacity* by a speech language therapist with expertise in experimental phonetics and who was not involved in data gathering and was blind to the participants' demographics and clinical status.

Acoustically, the waveform, spectrogram, pitch, intensity, and the formants of each sustained vowel were visually observed using the *Praat* 5.1 software downloaded from http://www.praat.org. [183]

The vowel /a/ mean and standard deviation F_0 (Hertz, Hz), jitter (local, %) and harmonic-to-noise-ratio (dB) were analyzed with a moving window with at least 1-second using voice report in the *Praat* software.

The following parameters were analyzed: a) *Respiratory support for speech*. Duration (seconds) was measured as the total period between the onset and offset of each sustained vowel /a/ and the breath(s) during speech in the sentence 'A Maria comprou-me um mapa de papel branco'; b) *Voice (pitch) quality*. The average F_0 (Hertz) was analyzed in all vowels in the two moments. Vowel /a/ was perceptually analyzed by a speech language therapist for pitch and loudness level along the production (mainly high or low); c) *Voice (pitch) stability*. The assigned acoustic parameters were: Pitch breaks (no pitch contour) time (seconds) and jitter (local, %). Vowel /a/ was perceptually analyzed by considering the pitch and loudness stability (maintained, increased, decreased or uncontrolled); d*) Voice variability.* Variability was considered as speech F0 standard deviation in Hz in the sentence (Sentence F_0SD). At baseline (MED OFF) the F_0SD (Hz) was also analyzed; *e) Speech rate.* Speech rate of the sentence 'A Maria comprou-me um mapa de papel branco' [Mary bought me a map of white paper], total number of orthographic syllables divided by total time duration (including pauses).

2.4 Statistical analysis

Descriptive statistics of demographic, clinical, and therapeutic data were provided for continuous (median and interquartile range [IQR, 25th–75th percentile]) and categorical (count and percentage) variables.

Voice and speech characteristics at baseline (MED OFF) of LSPD patients, considering men and women separately, were compared to the available normal values of healthy age-matched subjects, although no statistical analyses were performed.

The acute effect of L-dopa on voice and speech was calculated by comparing the median duration of the vowel /a/, average F_0, pitch breaks duration, jitter, SF_0SD, and speech rate between MED OFF versus MED ON conditions. Comparisons were made using the Wilcoxon's signed-rank test.

Spearman's rank correlation coefficient was used to assess the association between: a) respiratory support for speech, voice quality, voice stability, voice variability, speech and disease duration, and motor impairment (MDS-UPDRS-III) /axial motor impairment (sum of items 3.1, 3.10-3.12 of the MDS-UPDRS-III); b) speech rate and freezing (item 3.11 of the MDS-UPDRS-III).

Two group comparisons (women *versus* men) were performed using the Mann-Whitney U-test.

Reliability of analyses. To evaluate test-retest reliability of acoustic measurements the sustained vowel /a/ for an average F_0 was run twice. A satisfying test-retest reliability was found ($r=0.722$, $p<0.001$, Pearson test), only one single-speech-task cycle was performed for the definite acoustic measurements.

A P value <0.05 was considered significant. The analysis of the results was carried out by means of SPSS 21.0 (SPSS, Chicago, IL).

Results

Clinical data

Twenty-seven LSPD patients were recruited for speech and voice analyses. Three were excluded due to their inability to perform the required tasks (one anarthric patient and two due to severe dementia). Demographic and clinical data of the 24 LSPD patients are detailed in Table 1.

There were no differences in demographic or clinical variables between men and women (Table 1). Indeed, they presented similar MDS-UPDRS II-III-IV scores, axial signs score, SE and HY stages, although women had a slightly, but not statistically significant, worse HY stage, and more men were demented although not statistically significant (Table 1).

Table I. Demographic and clinical data

Patients data	LSPD (n= 24)	LSPD MALE (n=14)	LSPD FEMALE (n=10)	*p - value*
Age (yrs)	79 [71.5-81.7]	77.5 [70.7-81.2]	79 [73.5-85]	ns
Age at disease onset (yrs)	64.5 [54.5-69.5]	62.5 [55-67]	65 [51.5-71.5]	ns
Disease duration	14.5 [11-15.7]	13.5 [8.7-17]	15 [11.7-17.2]	ns
Education (yrs)	4 [4-11]	4 [4-12]	5 [4-10.5]	ns
S&E (ON/OFF)	40/35 [40-40.7 / 22.5-40]	40/30 [40-40/ 40-40]	40/30 [27-50 / 17.5-50]	ns
HY (ON/OFF)	4 [2-4] / 4 [2-4.75]	3 [2-4] / 3 [2-4]	4 [4-5] /4 [4-5]	ns
PDD (n (%))	14 (58%)	10 (71%)	4 (40%)	ns
MMSE **MMSE (demented/non-demented)**	22.5 [21.2-25] 22 [17-23.7] / 25 [23-26.7]	22.5 [22-24.2] 22 [21.7-24.2] / 23 [22.2-25.2]	22.5 [16-27.2] 17 [13-19.5] / 27 [25-28.5]	ns
LEDD (mg)	1037 [902-1272]	1100 [990-1303]	905 [742-1257]	ns
MDS-UPDRS-II	31 [27-38]	32 [29.2 – 38.5]	30 [20.5-38]	ns
MDS-UPDRS-III (MED ON/MED OFF)	50 [40-54]/64 [52-77]	50 [42.5-55.2]/61[53-76]	50 [37.5-62.5] /64 [48-79.5]	ns
Axial sign (MED ON/MED OFF)	8 [6-13] /10 [7-13]	8 [6-13]/10 [7-13.2]	8 [6.5-12]/ 10 [7-13.5]	ns
MDS-UPDRS-IV	4 [2-9.5]	5 [2-8.5]	4 [0-11.2]	ns

Values are presented as median [IQR, 25th–75th percentile] if no otherwise specified; ns: not significant. LEDD: L-dopa equivalent daily dose; PDD: Parkinson's disease with dementia; MMSE: mini mental state examination. S&E: Schwab and England score; HY: Hoehn Yahr Stage; ns: non-significant; P value is the results for male vs. female scores' comparison.

Baseline (MED OFF) voice and speech characteristics

No differences were found between men and women for breath support and voice stability at baseline (MED OFF) (Table 2). Voice quality differed between men and women at baseline, although this difference has been noticed in vocally healthy subjects (gender effect) and the values were also similar to vocally healthy subjects (JA, 2015) (Table 2). Values of respiratory breath support [184]and pitch break time[183]of LSPD patients appeared worse when compared to the normal values of healthy age-matched subjects, stratified for gender (Table 2). Mean jitter values were in the normal range (Table 2), although results were borderline for men and SD showed a tendency for higher values.[185] In contrast, F_0SD [186]was in the normal range (Table 2). However this result was partially expected as we use a very syntactically simple sentence.

A positive moderate correlation was found between disease duration and voice quality (R=0.51; p=0.013) and a negative one with speech rate (R= - 0.55; p=0.008). Motor impairment (MDS-UPDRS-III) had a moderate significant correlation with respiratory support for speech (R= -0.43; p=0.045) and pitch break time (R= -0.565; p=0.006). No correlations were found between voice and speech features and axial motor impairment, neither between speech rate and freezing. When analyzing by gender (men and women separately) such correlations were partially maintained: a) voice quality and disease duration: men [R=0.5; p=0.079] and women [R=0.36; p=0.2]; b) speech rate and disease duration: men [R= -0.7; p=0.003)] and women [R= -0.2; p=0.5]; c) respiratory support for speech and MDS-UPDRS-III: men [R=0.64; p=0.017] and women [R= -0.7; p=0.029].

Table 2. Voice and speech baseline features

	PD Patients (N=24)		**Normal value**	
Respiratory support for speech Vowel duration (sec)	5.8 [4.4-11.5.8]		22.97 (1.1) ^	
Voice stability				
Pitch break time (sec)	1.24 [0.2-2.6.1]		NA*	
Jitter (%)	0.8 [0.5-1.1]		≤ 0.5-1%	
Voice variability F_0SD (Hz)	2.4 [1.6-4]		2-4Hz	
Voice quality (Hz) F_0	MALE **(N=14)**	FEMALE **(N=10)**	MALE	FEMALE
	125 [104-152]	202 [160-226.8]	128 (36)**	198 (44)**

Values for LSPD patients are presented as median [IQR, 25th–75th percentile]. Values for healthy subjects are presented as mean (SD), as reported in literature [184-187]. F_0: fundamental frequency; F_0SD: fundamental frequency standard deviation; NA*: not available (healthy voices should have no trouble in maintaining voicing during a sustained vowel. Thus is 0% of voice breaks. No standard values are available). ^: normal value for vowel duration are referred to a healthy population aged between 71 and 80 years old. **: normal value for voice quality are referred to a healthy population aged between 55 and 80 years old.

L-dopa acute challenge test

No differences between men and women were found when comparing motor, voice, and speech variables during both MED OFF and MED ON, except for voice quality (F_0), as was expected (see Table 2 for voice characteristics of healthy subjects). Thus, further analyses were carried out by taking into consideration the whole LSPD sample and not stratifying by gender.

Motor response. The median L-dopa dose for the test was 375 mg (IQR: 277-375). The median MDS-UPDRS-III score was 64 (IQR: 52-77) in MED OFF and 50 (IQR: 40-54) in MED ON, with a significant median improvement of 20% (IQR: 11.5% - 32%) ($p<0.001$) (Table 2). Sub-analysis of MDS-UPDRS-III scores for axial signs showed a significant median improvement after L-dopa intake for all the sub-items, except speech (Table 2). 3 patients (12.5%) had mild dystonic dyskinesias in MED OFF, while 12 (50%) presented slight-moderate choreic dyskinesias in MED ON.

Voice and speech response. None of voice and speech variables changed significantly after L-dopa intake (Table 2).

Equally, separate analysis of non-demented and demented patients showed no modification of speech and voice variables following L-dopa intake.

Table 3. Levodopa challenge test

LSPD patients (N= 24)			
	MED OFF	MED ON	p - value
MDS-UPDRS-III	64 [52-77]	50 [40-54]	<0.001
Speech	2 [1-3]	2 [1-3]	0.83
Freezing of gait	3 [1-4]	2 [0-3]	<0.05 (0.01)
Postural Stability	3 [2-4]	3 [2-3]	<0.05 (0.014)
Gait	3 [2-4]	3 [2-3]	<0.05 (0.01)
Axial Signs	10 [7-13]	8 [6-13]	<0.05 (0.01)
HY	4 [2-4.75]	4 [2-4]	0.7
mAIMS	0	1 [0-6.75]	0.04
Voice Respiratory support for speech Vowel duration (sec)	5.8 [4.4-11.5]	7 [3.6-10.6]	0.6
Voice stability			
Pitch break time	1.2 [0.2-2.6]	0.8 [0.07-2.5]	0.9
Jitter	0.8 [0.5-1.1]	0.7 [0.4-1]	0.5
Voice quality F_0	154 [123-209]	162 [147-203]	0.2
Voice variability SentenceSFoSD	31 [19-51]	29 [20-40]	0.5
Speech rate	5 [3.6-5.6]	5 [4.2-5.7]	0.2

Values are presented as median [IQR, 25th–75th percentile]. Statistical significant results are in bold. Axial Signs: sum of item 3.1, 3.10-3.12 of the MDS-UPDRS-III. P – value is the results of MED OFF versus MED ON scores. mAIMS: Modified Abnormal Involuntary Movement Scale.

Discussion

The purpose of this study was to explore the L-dopa response of speech in the late stage of PD. In order to do this a population of LSPD patients underwent an L-dopa challenge while performing specific vocal tasks during both MED OFF and MED ON conditions. No effect of L-dopa was found on speech and voice by means of both automated analysis and clinical evaluation, although patients had a moderate positive motor response, even present for some axial signs, with the exception of speech. Such a discrepancy in L-dopa responsiveness between speech and other axial signs has been reported only in one previous speech study in advanced PD patients [179]and suggests that speech together with balance and postural problems could be listed among L-dopa resistant axial sign appearing with disease progression.

Despite not performing a case-controlled study, by comparing MED OFF speech and voice characteristics of our patients with normative values of the general population we found a severe impairment of respiratory support for speech and voice stability, as already reported elsewhere. [171, 177] We chose to make this comparison in the MED OFF condition because it more accurately reflects the parkinsonian state of patients. Rigidity associated with PD can often lead to disruption of respiratory processes, which serve to generate air pressure for speech. [175] Respiratory support for speech may be measured through vowel prolongation, and a decrease by an average of fifty percent in vowel prolongation has been reported for PD patients when compared to normal healthy speakers. [175] Among our LSPD patients, vowel prolongation was more affected, even in the absence of dyskinesias that can affect respiratory control. [176] Equally, voice stability, i.e., ability to maintain a consistent voice during a stable/sustained vowel with laryngeal muscle effort, is impaired in MED OFF, as shown by an increase in pitch break time and the tendency for jitter increment. Moreover, a tendency for worsening voice quality and speech rate was highlighted with disease duration. Voice quality and voice variability values in MED OFF were in the normal range although the most plausible cause for this finding is methodological, which might have resulted in falsely normal values for voice quality and variability: we have chosen a declarative sentence for voice variability analysis that is syntactically too simple to capture this feature; equally, we assessed voice quality using mean F0 instead of SDF0 which is usually more appropriate but not possible to analyze in our patients due to the technical quality of the recordings. Interestingly no correlations were found between speech rate and freezing. This data is apparently in contrast with the recent findings of Ricciardi and colleagues that showed lower scores in the articulation, intelligibility, rate/prosody section

of the Dysarthria Profile in PD patients with freezing of gait (FOG), as assessed by the New Freezing of Gait questionnaire, if compared to PD patients without FOG. [188]However in our study different methodological measures have been adopted in order to assess both speech rate and FOG. Moreover, Ricciardi and colleagues included younger PD patients, belonging to several HY stages, thus a more heterogeneous PD sample, scarcely comparable to our LSPD patients.

Our sample of LSPD patients still presented moderately good motor response to L-dopa (20% of the MDS-UPDRS-III) when compared to our previous report, and the frequency of dementia was slightly lower (52%). [78]The exclusion of patients who could not speak at all or who could not properly understand the tasks would have surely created bias. Thus, our sample may represent a subset of LSPD patients who present a slightly better clinical state compared to other reports. [54, 56]Nevertheless, even if an influence of dyskinesias on speech performance cannot be excluded[176], speech showed no improvement after L-dopa intake, whether it was measured clinically or with automated analysis that explored the respiratory support for speech (vowel duration), voice stability, variability and quality, and speech rate. De Letter *et al.* evaluated respiratory features among 25 non-demented PD patients during an L-dopa challenge and reported a slight improvement of sustained vowel phonation. [176] However, due to the clinical differences with our sample, i.e., older patients with longer disease duration and worse L-dopa response, these results may not be comparable with those published by De Letter *et al*. Concerning voice stability and variability, if we assume that hypokinesia of the voice apparatus is the major pathological mechanism of monopitch speech in PD [189, 190], F_0SD should improve after L-dopa intake and should decline further during the disease course. However, data on voice stability/variability improvement after L-dopa are inconsistent, and previous reports have also failed to show a response of F_0SD or jitter to dopaminergic therapy. [177, 180, 191] This finding may be related to the usual worse response of axial muscles to levodopa.

A lack of improvement in speech quality (F0) and speech rate after L-dopa or apomorphine has already been described in earlier PD stages. [177, 179, 180, 192] We report similar data in LSPD patients, although we have to consider that our patients did not present with a severe impairment of voice quality in MED OFF. Thus, an improvement would not be expected. A slight improvement of speech rate after L-dopa intake has been found in only 9 PD patients with optimal L-dopa responsiveness and a non-severe impairment of speech at baseline, as assessed by the UPDRS-III.[191] However, Ho *et al.* concomitantly reported on a decay of rate

improvement during the speech testing tasks.[191] Thus, it is likely that improvement in speech rate is not maintained during the tasks.

Several factors can contribute to the lack of speech and voice responsiveness to L-dopa in PD patients, especially in the late disease stage.

Speech production is essentially a series of skilled motor gestures that require upstream central coordination mediated by cerebral networks for speech production. Indeed, the globus pallidus (GP) produces a phasic burst of activity that triggers the supplementary motor area (SMA) neural discharge, allowing cortical motor set for movement preparation and subsequent execution. [191] In PD, the impairment of GP activity alters those mechanisms, resulting in diminished movement amplitude and impairment of movement sequencing. Such a process affects speech production as well as body movement, and a correlation between speech hypophonia/speech intensity and severity of body bradykinesia has been suggested. [191] L-dopa has been shown to have an effect on preparatory motor set, resulting in hypokinesia improvement, but failed to affect movement sequencing.[193] Likewise, concerning speech, while still controversial, a few studies have reported on a slight L-dopa positive effect on loudness (speech intensity), intonation (speech variability) and speech rate [177, 191] at least in early-advanced PD stages. Conversely, speech stability and variability seem to be definitively impervious to dopaminergic therapy. [189,194] Interestingly, and contrary to previous suggestions, we did not find neither an improvement of speech intensity or rate with L-dopa, nor a correlation between speech and voice severity and motor symptoms that still respond to L-dopa, namely bradykinesia and rigidity. These findings may support a non-dopaminergic involvement in speech neurocircuitry as already supposed in earlier disease stages[192], and this is even more likely in late stage PD.[1] Alternatively, a higher dose of L-dopa could be needed to improve speech, as is often the case with gait dysfunction. The usual absence of significant rigidity in late stage patients[54, 78]may also have contributed to the lack of correlation between speech intensity and motor impairment. Furthermore, we have to consider that a loss of striatal responsiveness is related to disease progression, and is likely responsible for a decrease or loss of clinical response to dopaminergic therapy of several motor symptoms[76], which also probably affects speech responsiveness. Finally, motor speech production also depends on the appropriate function of peripheral nervous system.[172] Dysfunction of speech articulation may also be partly attributed to muscular denervation and atrophy, resulting in respiratory muscles impairment whose function does not improve with L-dopa as recently shown in a sample of PD

patients in HY 2-4.[195] Such muscle impairment is presumably even more severe among older PD patients who have a worse motor status as our sample.

Our findings highlight the need for alternative non-dopaminergic/non-pharmacologic treatments to improve communication of LSPD patients. For instance, the Lee Silverman Voice Treatment (LSVT) has shown some efficacy in the treatment of voice and speech problems of PD patients (Pinto et al., 2004). However, its applicability to LSPD patients should be verified due to the level of collaboration that it requires and the degree of disability of those patients.

Study limitations

Some limitations of our study must be highlighted. Due to the clinical disability of LSPD patients, recordings were performed in a home environment and not in a laboratory setting. This implied accepting samples varying in context, over different time periods, and recorded in non-standard environments. Nevertheless, the quality and reliability of the recordings were evaluated by a speech language therapist. Patients' disabilities can also have influenced choice of tasks. For instance, we selected a simple task for voice variability assessment, which was probably not sensitive enough to detect L-dopa effect in voice/intonation variability, or voice variability defect at baseline. Finally, clinical assessment of patients was not blinded. However, there was concordance between clinical and automated assessments of speech.

Conclusions

To the best of our knowledge, this is the first report on L-dopa response of speech and voice in a sample of LSPD patients by means of both a clinical rating scale and automated analysis. Speech is severely affected among LSPD patients, as already reported for PD patients in earlier disease stages. [166, 169]

Although L-dopa still had some effect on motor performance, including some axial signs, we found no improvement in speech and voice. Clinical management and research should consider the applicability of non-pharmacological treatments for speech and voice impairment among LSPD patients.

CHAPTER 5: Dysarthria management in subthalamic deep brain stimulated Parkinson's disease patients

Is lowering stimulation frequency a feasible option for subthalamic deep brain stimulation in Parkinson's disease patients with dysarthria?

Abstract

Background: The long-term effect of subthalamic nucleus deep brain stimulation (STN-DBS) on dysarthria is variable and sometime detrimental. A transient beneficial effect of low-frequency stimulation (LFS) has been reported.

Objective: to investigate the effect of LFS on speech in STN-DBS treated PD patients and to verify whether the benefit is maintained over time.

Methods: a case-control study comparing 10 PD patients (Group A) with severe speech impairment (MDS-UPDRS item 3.1 $\geq$ 3) with 10 PD patients (Group B) with mild speech impairment (MDS-UPDRS item 3.1 $\leq$ 2) , all submitted to STN-DBS. Patients were tested in: MED OFF/STIM OFF, MED OFF/STIM ON (130Hz, high frequency stimulation [HFS]), MED OFF/STIM ON (60Hz - LFS) and MED ON with both HFS and LFS. The following was assessed in all conditions: voice (average and standard deviation fundamental frequency and jitter), speech (articulatory diadochokinesis [DDK], pitch variability, rate and intelligibility) and motor performance (MDS-UPDRS-III).

Results: LFS compared to no stimulation and HFS, in the absence of L-dopa effect, significantly improved DDK and speech intelligibility in Group A. Comparing LFS to HFS, with concomitant L-dopa intake, there was a significant improvement of speech intelligibility in both groups.

Five Group A patients opted to maintain LFS. After six months, four were still at 60-80 Hz stimulation. Speech benefit was maintained but treatment adjustments were required.

Conclusions: LFS may offer both an immediate and long-lasting improvement of speech in STN-DBS patients with severe speech impairment. Nevertheless, its effect on motor symptoms may not be preserved over time.

Introduction

Speech disorders affect nearly 70% of Parkinson's disease (PD) patients.[172] Parkinsonian hypokinetic dysarthria is characterized by hypophonia and dysprosody that worsen with the progression of the disease due to breath, phonation and articulation dysfunction. [172] Deep brain stimulation of the subthalamic nucleus (STN-DBS) is a common adjunct surgical treatment for the motor symptoms of PD, typically recommended for patients who have developed motor fluctuations and entered the advanced disease stage.[196] Although STN-DBS has been shown to be highly effective for cardinal motor symptoms associated with PD[196], its effects on speech are variable, multifactorial and sometime detrimental.[197] After one to five years since STN-DBS, in spite of an improvement of voice tremor and loudness, speaking pitch variability[198], articulatory diadochokinesis[199], speech rate and intelligibility tend to deteriorate, depending also on electrodes position and pre-operative speech characteristics.[200] Indeed, the most significant predictive factors for deterioration of speech intelligibility are lower preoperative speech intelligibility, longer disease duration, and a medially placed left active electrode contact.[200]

However, the role of STN-DBS in parkinsonian dysarthria and its management are still a matter of debate. A beneficial acute effect of low frequencies stimulation (LFS) and high voltages on speech intelligibility and laryngeal coordination has been suggested in few small-sampled studies, with no follow-up data available.[201, 202]

Our primary aim was to evaluate the modifications of speech parameters to an acute stimulation challenge with LFS in STN-DBS treated PD patients with mild/severe speech impairment and to assess whether the benefit obtained with LFS, when present, could be maintained over time without parkinsonian aggravation. As secondary aim, we also explored the concomitant acute effect of levodopa (L-dopa) and LFS on speech and voice.

Patients and methods

Study protocol and patient recruitment

We performed a case-control study, comparing 10 PD patients (UK Brain Bank criteria[77]) with with severe speech impairment (([Movement Disorder Society (MDS)-sponsored revision of

the Unified Parkinson's Disease Rating Scale (UPDRS) item 3.1 ≥ 3[49], Group A) versus 10 PD patients with mild speech impairment (MDS-UPDRS item 3.1 ≤ 2, Group B) , all treated with STN-DBS for at least 3 years. Groups were matched for gender, age and age at disease onset. PD patients with dementia[83] were excluded. The Local Ethical Committee approved the study and all patients provided a written informed consent.

Neurosurgical procedure

STN-DBS surgery was performed as previously described with quadripolar leads (electrode model 3389; Medtronic, Minneapolis, MN), with a bilateral lead implantation based on magnetic resonance imaging (MRI) / computed tomography (CT) image fusion for anatomical targeting, intraoperative electrophysiological recording and microstimulation.[203] Postoperative MRI was performed to confirm electrode positioning and to exclude surgical complications in all patients.

Assessment of patients

Patients were assessed in the following conditions: Medication OFF/Stimulation ON (M-Off/S-On) - 130 Hz, M-Off/S-Off, M-Off/S-On_60 Hz, M-On/S-On_60 Hz and M-On/S-On_130 Hz. M-Off condition was reached after at least 12 hours the last L-dopa intake. Each stimulation condition was maintained for at least 60 minutes before patient's assessment. For the M-On condition, each patient was evaluated 45-60 minutes after the intake of the usual morning L-dopa dose.

The equivalent voltage for LFS was calculated for each patient using the total electrical energy delivered (TEED) formula: TEED (1s) = voltage^2 x frequency x amplitude/impedance.

During each condition we assessed: (a) speech and oromotor performance by means of digital recordings of a steady vowel production (vowel /a/, repeated three times), an oral reading performance and a set of repetitive syllables (/pa/, /pata/, /pataka/) for all patients; (b) motor performances by means of the MDS-UPDRS part III and the Timed up and go test (TUG) ; (c) dyskinesias severity by means of the Modified Abnormal Involuntary Movement Scale (mAIMS); (d) the Clinical Global Impression Improvement Scale (CGI-I). If CGI-I during M-On/S-On_60Hz vs. M-On/S-On_130Hz) was ≤3 (slight to great improvement), the patient was maintained on LFS and follow-up visits were scheduled after two weeks (clinical assessment) and six months (clinical and automatic speech assessment). At baseline, patients were also

assessed by means of: a) the Quality of life in the dysarthric speaker questionnaire (QoL-DyS, Italian version)[204]; b) the New freezing of gait questionnaire (NFG-Q)[205]; c) the MDS-UPDRS part I-II and IV. L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversion.[81]

All voice and speech samples were recorded in a quiet hospital room using a tabletop unidirectional microphone (Fame, MS- 1800S) attached to a preamplifier (M-Audio Fast Track Pro, preamp, USB) and a desktop computer running *Audacity software version 2.1.2* (Free software Foundation Europe, Hamburg, Germany). Five separate perceptual files were completed with all the stimuli presented at the same sound pressure levels and with a 500 ms silence between single words and sentences.

Data analysis

All speech samples were copied to a computer (down sampled to 24 kHz, 16 bits, mono), edited into individual files and screened for extraneous noise using Audacity by a speech language therapist (SLT) who was blind to the participants' demographics, stimulation, and clinical status. Acoustically, the waveform, spectrogram, pitch, intensity, and the formants of each sustained vowel were visually observed using the Praat 5.1 software downloaded from http://www.praat.org. The vowel /a/, F0 (Hz) and jitter (local, %) were analysed with a moving window with at least 1-sec using voice report in the Praat software.

The following parameters were analysed: (a) Voice (pitch) quality: the average fundamental frequency (F0) in Hertz; (b) Voice (pitch) variability: the F0 SD (standard deviation); (c) Voice (pitch) instability: jitter (local, %). All parameters were analysed in all vowels in the three moments; (d) Speech rate (syllables/sec of the first and the last paragraph of a phonetically balanced text, of respectively 46 and 41 syllables); (e) Speech intelligibility, measured as: (i) the percentage of words from a list of 50 words correctly understood by two independent SLT blinded to patients' conditions; (ii) a VAS scale (from zero to 10, being 10 the best score) evaluated by a blinded SLT, who scored speech intelligibility during a text reading; (f) Articulatory diadochokinesis (DDK): the number of syllables, /pa/ (alternating motion rate, AMR, articulatory movement of the jaw combined with the lips), /pata/ and /pataka/ (sequential motion rate, SMR, articulatory movement of the jaw combined with the lips and the anterior and posterior parts of the tongue), at a fast rate during 5 sec each.

Statistical analysis

Clinical and demographic characteristics were summarized as mean ± standard deviation or percentages, as appropriate. Two group comparisons were performed using Mann-Whitney U-test. The acute effect of LFS was calculated by comparisons between different therapeutic conditions using the Wilcoxon's signed ranked test. All the analyses were performed with SPSS 23.0 (SPSS, Chicago, IL) using two-tailed p-values with a level of significance of 0.05.

Results

Demographic, clinical and therapeutic data of the patients are detailed in Table 1.

Table 1. Demographic, clinical, therapeutic and speech characteristics of DBS patients

	Group A (n=10)	**Group B (n=10)**	**p-values**
Age (yrs)	65.3 ± 6.1	63.5 ± 5.7	ns
Women (n/total (%))	3/10 (33%)	3/10 (33%)	ns
Age at disease onset (yrs)	46.3 ± 6.6	43.6 ± 7.1	ns
Disease duration (yrs)	19 ± 5.2	19.9 ± 4.9	ns
Age at DBS (yrs)	58.4 ± 5.7	56.7 ± 8.1	ns
Months after DBS	82 ± 42	81.1 ± 36.7	ns
LEDD before surgery (mg) **LEDD after surgery (mg)**	1180.7 ± 436 812 ± 610	1045 ± 337 680 ± 420	ns ns
Stimulation Voltage **R_STN/L_STN at 130 Hz** **R_STN/L_STN at 60 Hz**	 3.4 ±0.7 / 2.8 ± 08 4.8 ±1 / 4.2 ±1	 3.4 ±0.5 /3.4 ±0.4 5 ±0.7 5.1 ±0.6	ns
Electrodes position * **Ventral n (%)**	4 (40%)	4 (40%)	ns

MMSE	27 ± 1.7	28.6 ± 1.2	ns
NFG-Q	13.8 ± 7.4	6.4 ± 8.1	0.048
SE (ON)	81 ± 11	86 ± 6.9	ns
MDS-UPDRS-I **MDS-UPDRS-II** **MDS-UPDRS-III** **MDS-UPDRS-IV**	11.3 ± 3.6 22.5 ± 6.1 27.2 ±9.8 5 ± 3.6	11.4 ± 5 18.2 ± 4 22.2 ± 9.7 1.6 ± 2.2	ns ns ns 0.035
QoL-DyS, total score Speech characteristics Situational difficulty Compensatory strategies Perceived reactions of others	49 ±22.6 19.2 ± 7.3 14.8 ± 6.4 5.3 ± 7.8 9.5 ± 9.5	16.2 ± 16 7.2 ± 6 4.4 ± 2.9 2.6 ± 6.1 2 ± 3.3	<0.01 <0.01 <0.001 ns <0.05

Group A: PD patients with severe speech impairment (MDS.UPDRS ≥ 3); Group B: PD patients with mild speech impairment (MDS.UPDRS 2.1 ≤ 2); ns: not significant; Values are presented as mean ± SD, if not otherwise specified. LEDD: levodopa equivalent daily dose; R_STN: right subthalamic nucleus; L_STN: left subthalamic nucleus; NFG-Q: New freezing of gait questionnaire; MMSE: Mini mental state examination; SE: Schwab and England Scale (MED ON/STIM ON); QoL-DyS: Quality of life in the dysartrhic speaker questionnaire (total score range: 0-160, higher score = higher impact); (*): Electrode position was been classified as "ventral" if the active contact was one of the two most ventral contacts.

Comparing men and women there were no differences in motor, voice, and speech variables in all therapeutic conditions, except for voice quality (average F_0), that was higher among women, as expected. Thus, further analyses were carried out without stratifying by gender.

We describe below speech, voice and motor parameters changes in different therapeutic conditions.

M-Off/S-Off condition. With no L-dopa and no stimulation, voice instability (Jitter) and DDK of all patients was worse if compared to vocally healthy subjects' values with same age (Table

2).[186, 187, 206] No differences were found for average F_0 analysing men, women and Groups separately [(data not showed; Group A: man 138±30; women: 172±20; Group B, man: 128±29; women: 178±30)]. DDK and speech intelligibility was worse in Group A if compared to Group B ($p< 0.05$).

Effect of HFS. HFS (M-Off/S-Off vs M-Off/S-On 130Hz) did not significantly change voice and speech parameters, apart for a slight improvement of DDK and an increment of F_0 in Group B (Table 1). Concomitantly, motor performances significantly improved by 40±25% and 45±12% at the MDS-UPDRS-III in Group A and B, respectively and an improvement of TUG (Table 2) was noted.

Effect of LFS. LSF (M-Off/S-On 60Hz vs M-Off/S-Off) significantly improved SMR (syllable /pata/) in both groups, SMR (syllable /pataka/) and speech intelligibility for sentences in Group A and speech intelligibility for words in Group B (Table 2). Concomitantly motor performances significantly improved by 39±22% and 42±12 % at the MDS-UPDRS-III in Group A and B, respectively with improvement of TUG only in Group A (Table 2).

Effect of LFS vs HFS without L-dopa. Comparing condition M-Off/S-On 130 Hz vs. M-Off/S-On 60 Hz, we found: a) a statistically significant improvement of DDK (syllable /pataka/), speech intelligibility for sentences and MDS-UPDRS item 3.1in Group A; b) An improvement not reaching statistical significance of voice instability (jitter%) in group B; c) no changes for voice and speech parameters in Group B with level of voice instability maintained within acceptable values (<1%) in both conditions; d) no significant changes in motor performances or dyskinesias development in both groups (Table 2).

Effect of LFS vs. HFS with L-dopa. Comparing condition M-On/S-On 60Hz vs. M-On/S-On 130Hz, we found: a) a statistically significant improvement of speech intelligibility for sentences in both groups; b) a significant reduction of speech rate of the first paragraph in Group B; c) no significant changes in motor performance or dyskinesias development in both groups (Table 2).

L-dopa effect. No significant speech modification was revealed after L-dopa intake with both stimulation frequency (M-Off/S-On 60Hz vs. M-On/S-On 60Hz and M-Off/S-ON 130Hz vs. M-On/S-On 130Hz), with the exception of a slight worsening of DDK in Group B (Table 2).

The TEED was held constant and no clinical manifestation of current diffusion was observed.

Table 2. Speech and voice response to LFS, HFS and L-dopa in combination with stimulation

	Group A (n=10)							
	M-Off/S-Off (A)	M-Off/S-On 130Hz (B)	M-Off/S-On 60Hz (C)	M-On/S-On 60Hz (D)	M-On/S-On 130Hz (E)	Stimulation effect P – values a) LFS: A vs. C b) HFS: A vs. B	Medication effect P-value a) LFS: C vs. D b) HFS: B vs. E	Frequency effect P – values a) M-Off: C vs. B b) M-On: D vs. E
Voice quality Average F_0	148.6 ±32.5	150.1 ± 47.5	154.6 ±29.1	158.4 ± 30.8	144.1 ±45.6	a) ns; b) ns;	a) ns; b) ns;	 a) ns; b) ns;
Voice variability F_0 SD	7.1 ± 6.1	17.6 ± 6.1	9.1 ±8	10.9 ± 9.4	12.3 ± 7.3	a) ns; b) n;	a) ns; b) ns;	a) ns; b) ns;
Voice instability Jitter	1.2±1.3	1.1± 1.2	0.9±0.5	0.8 ± 0.5	0.9 ±0.7	a) ns; b) ns;	a) ns; b) ns;	a) ns; b) ns;
Speech rate First paragraph Second paragraph	 4.5 ±1.4 3.9 ±1.1	 4.4 ± 1.1 4.1 ± 1.3	 4.5 ± 1.2 4.4 ±1.4	 4.4 ± 1.2 4.1 ± 1.1	 4.4 ± 1.2 4.4 ± 1.1	 a) ns; b) ns; a) ns; b) ns;	a) ns; b) ns; a) ns; b) ns;	 a) ns; b) ns; a) ns; b) ns;
Oral diadochokinesis /pa/ /pata/ /pataka/	 2.9±1 1.8±0.7 1.3 ±0.5	 3.5±0.9 2±0.6 1.4 ±0.4	 3.2±0.7 2±0.5 1.6 ±0.4	 3.5±0.8 2.1±0.6 1.6 ±0.4	 3.2±1.3 2.4±0.5 ±0.2	 a) ns; b) ns; a) <0.05; b)ns; a) < 0.05; b) ns;	 a) ns;b) ns; a) ns; b) ns; a) ns; b) ns;	 a) ns; b) ns; a) ns; b) ns a)<0.05; b)ns
Speech intelligibility Word list (%) Sentence	 74.4 ± 20 5.6 ± 1.5	 82.6± 12.5 5.8 ±2	 91.5 ± 8.5 8.1 ± 1.3	 88.6 ± 6.8 7.5 ± 1.4	 80± 9.3 4.5 ± 2.2	 a) ns; b) ns; a) < 0.05; b) ns;	 a) ns; b) ns a) ns; ns;	 a) ns; b) ns; a)<0.05 b)<0.05
mAIMS	1.3 ± 1.3	1.5±1.5	1 ± 1.1	4.3 ± 4	5.1± 5.5	a) ns; b) ns;	a) <0.05; b) ns;	a) ns; b) ns;
TUG (sec)	25.1 ± 11.4	18± 8	15.8 ± 8.6	13.6± 7.1	15.8 ± 8.6	a) <0.05; b) <0.05	a) ns; b) ns;	a) ns; b) ns;
MDS-UPDRS-III	60.1 ± 15.1	33.8 ± 11.7	35 ± 11.5	22.2 ± 14.8	24.8 ± 9.1	a) <0.01; b) <0.01	a) <0.05; b) <0.05	a) ns; b) ns;
MDS-UPDRS item 3.1	2.4±0.5	1.8 ±0.4	2.3 ±0.8	1.5 ±0.5	2.2 ±0.6	a) ns; b) ns;	a) ns; b) ns;	a)<0.05 b)<0.05

	Group B (n=10)							
	M-Off/S-Off (A)	M-Off/S-On 130Hz (B)	M-Off/S-On 60Hz (C)	M-On/S-On 60Hz (D)	M-On/S-On 130Hz (E)	**Stimulation effect** P – values a) LFS: A vs. C b) HFS: A vs. B	**Medication effect** P-value a) LFS: C vs. D b) HFS: B vs. E	**Frequency effect** P – values a) M-Off: C vs. B b) M-On: D vs. E
Voice quality Average F_0 (Hz)	133.7 ± 28.1	150.3 ±39.2	152.4 ±39.2	156.3 ±33.2	152.7 ±40.1	a) <0.05; b) <0.05	a) ns; b) ns;	a) ns; b) ns;
Voice variability F_0SD	15 ±14.4	16.9 ± 12.3	13.4 ± 15.5	11.8±12.4	11.2± 15.4	a) ns; b) ns;	a) ns; b) ns;	a) ns; b) ns;
Voice instability (Jitter)	0.7±0.6	0.8±0.8	0.8±0.7	0.5±0.4	0.4±0.4	a) ns; b) ns;	a) ns; b) ns;	a) ns; b) ns;
Speech rate First paragraph Second paragraph	 4.2 ±1 3.8 ± 1	 4.5 ± 0.6 4.3 ± 1	 4.5 ± 0.7 4.3 ± 0.7	 4.5 ± 0.7 4.1 ±0.8	 4. 9 ±0.8 4.5 ± 0.6	 a) ns; b) ns; a) ns; b) ns	 a) ns; b) ns; a) ns; b) ns;	 a) ns; b) <0.05 a) ns; b) ns;
Oral diadochokinesis **/pa/** **/pata/** **/pataka/**	 3.9±0.7 2.1±0.6 1.5 ±0.4	 4.1±0.5 2.5±0.4 1.7 ± 0.2	 4.1±0.4 2.6±0.4 1.9 ±0.3	 3.7±0.7 2.5±0.5 1.7 ±0.3	 4.1±0.7 2.4±0.2 1.7 ± 0.2	 a) ns; b) ns; a) <0.05; b) <0.05 a) ns; b) ns;	 a) ns; b) ns; a) ns; b) ns; a) <0.05; b) ns;	 a) ns; b) ns; a) ns; b) ns; a) ns; b) ns;
Speech intelligibility Word list (%) Sentence	 84.5 ±11.8 8.5 ±1.5	 91.7 ± 4.6 8.4 ± 2	 90 ± 5 8 ± 3	 91.6 ± 3.4 9.2 ±0.8	 88.8 ± 9.6 7.7 ± 2.2	 a) < 0.05; b) ns; a) ns; b) ns	 a) ns; b) ns; a) ns; b) ns;	 a) ns; b) ns; a) ns; b) <0.05
mAIMS	0.7± 1.3	0.1 ± 0.3	0.4 ± 0.8	4.4 ± 5	3.3 ± 3.5	a) ns; b) ns;	a) <0.05; b) <0.05;	a) ns; b) ns;
TUG (sec)	22.1 ± 18.8	9.3 ± 4.1	12.3 ± 6.5	8.6 ± 2.3	9.1 ± 3	a) ns; b) < 0.05	a) <0.05; b) ns;	a) ns; b) ns;
MDS-UPDRS-III	61 ± 13	32 ± 8	35.6 ± 12.4	19.5 ± 8.5	20.9 ± 9.3	a) <0.01; b) <0.01	a) <0.01; b) <0.05;	a) ns; b) ns;
MDS-UPDRS item 3.1	1.3 ± 0.8	1.2 ±0.6	1.4 ±0.7	1.1 ±0.6	1.4±0.8	a) ns; b) ns	a) ns; b) ns;	a) ns; b) ns;

LFS: low frequency stimulation (60Hz); HFS: high frequency stimulation (130Hz); M: MED; S: STIM; Oral diadockocinesis: number of /pa/, /pata/, /pataka/5 seconds; Speech rate: syllables/sec. Available values for vocally healthy subjects with same age: Average F0 (men: 128± 36; women: 198±44); speech rate: 3-6 syllables/sec; DDK: 5-7 syllables/sec; Hz; Jitter: < 1%; For one patient of Group A speech intelligibility analysis by means of sentences reading was not possible as speech was not understandable. Two patients of Group A and one Group B patient did not tolerate M-Off/S-Off condition for more than 30 minutes due to the severity of motor symptoms.

Group B patients referred no subjective speech improvement with LFS in MED ON (mean CGI-I= 4) and none of them maintained LFS. Conversely, five Group A patients reported subjective speech improvement with LFS (CGI-I score was 2 for all but one patient who scored 3) and were maintained on LFS. Follow-up data of these patients were as follows (Figure 1):

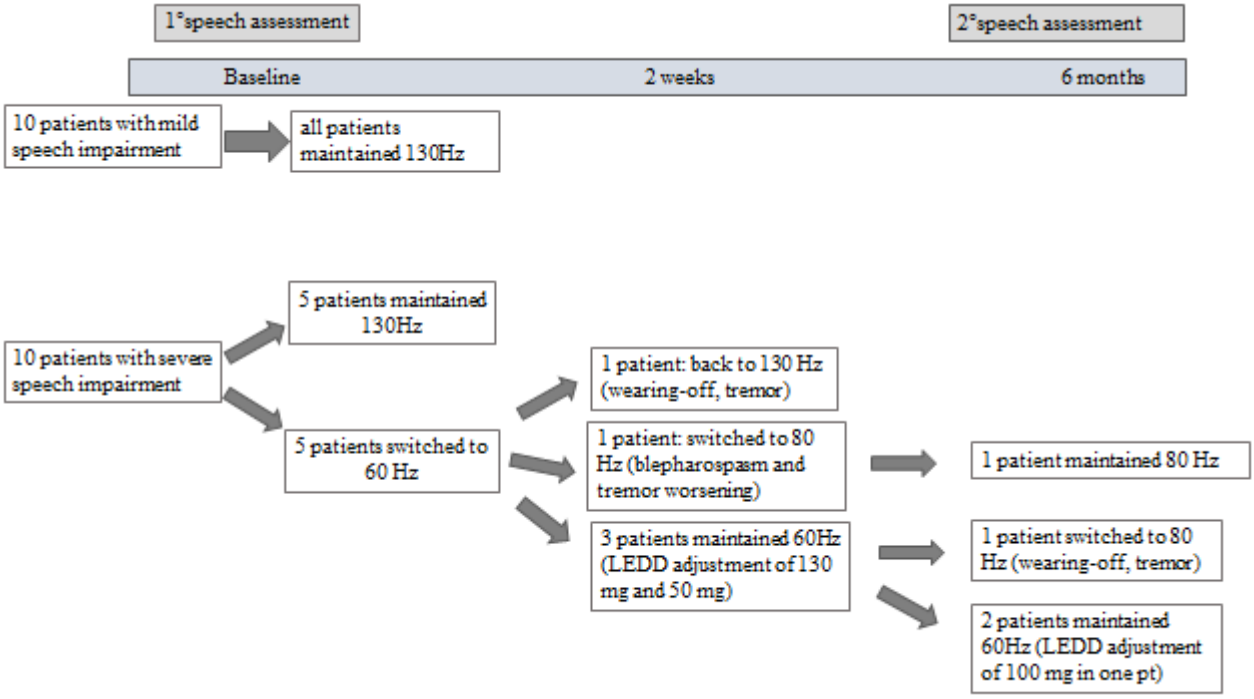
a) At two-week follow-up: one patient was switched back to HFS, due to wearing-off and severe resting tremor reappearance and one patient was switched at 80 Hz stimulation, due to worsening of tremor and blepharospasm appearance. Of the three patients who maintained the 60 Hz stimulation, two needed to increase L-dopa dose (delta LEDD: 130 mg and 50 mg, respectively) due to wearing-off and rest tremor reappearance;

b) At 6-month follow-up: the patient switched to 80 Hz after two weeks maintained such frequency. One other patient stimulated at 60 Hz frequency was switched to 80 Hz due to wearing-off worsening. Two patients maintained 60 Hz stimulation and an adjustment of oral therapy was required for one of them (delta LEDD: 100 mg).

Automatic speech analysis of the four patients who kept a frequency stimulation $\leq$ 80Hz revealed no significant difference of speech parameters and motor performance (p range: 0.07-0.2) if compared to baseline M-On/S-On 60 Hz.

As per inclusion criteria, Groups were matched for age, gender, age at disease onset and disease duration (Table 1). No differences were found for pre- and post-surgical LEDD, MMSE, STN-DBS treatment duration (almost 7 years), voltage intensity and MDS-UPDRS part I-II-III. Conversely, Group A had a slightly worse MDS-UPDRS-IV and NFG-Q scores and a more severe QoL-DyS score (Table 1).

Figure 1. Follow-up of patients who maintained a low frequency stimulation.



LEDD: levodopa equivalent daily dose.

Discussion

Hypokinetic dysarthria can severely impact PD patients QoL and speech worsening can counterbalance the motor benefits of STN-DBS. [207, 208] The management of speech impairment of STN-DBS treated patients remains particularly challenging. Indeed, several clinical and therapeutic factors can influence speech outcome, such as the pre-operative patient axial impairment, the lateral or medial electrode position[200], the concomitant effect of L-dopa and stimulation due to their effect on dyskinesias and respiratory control[176, 209] and the disease progression. Herein we assessed the acute effect of LFS on PD patients with different grades of speech impairment. LFS improved speech intelligibility both in the absence of L-dopa effect, and with concomitant L-dopa intake, among patients with severe speech impairment, chronically stimulated with conventional HFS. Among patients with mild speech impairment,

a statistically significant improvement of speech intelligibility was also detected with LFS, though it was not clinically meaningful, as expected.

Although the results failed to indicate any statistically significant difference in voice instability, under the effect of LFS, some trends were noted. Indeed, under LFS effect the voice instability (jitter magnitude) decreased from indices considered pathological to normal in patients with severe dysarthria, if compared to no stimulation and HFS without L-dopa. Considering that a steady vowel production elicits a stationary process of the articulatory-laryngeal system[175], reflecting the sound produced by the vocal folds it may be speculated that LFS contributes to a better neuromuscular vocal fold control of phonation in mild dysarthria patients but the effect is more evident in severe dysarthria.

LFS compared to no stimulation and to HFS, in the absence of L-dopa effect, significantly improved DDK among patients with mild and severe dysarthria alike and speech intelligibility for sentences only in patients with severe dysarthria. LFS without versus with L-dopa intake significantly worsened DDK among PD patients with mild dysarthria and induced no improvement among those with severe dysarthria. LFS versus HFS, with L-dopa intake, reduced speech rate in PD patients with mild dysarthria and improved speech intelligibility in both groups, although the improvement of intelligibility was clinically evident only for patients with severe dysarthria. Interestingly, HFS did not have an acute detrimental effect on speech in both Groups. However, a detrimental acute effect of HFS on voice instability and speech intelligibility was found at baseline in the two patients who maintained LFS 60Hz at follow-up (data not showed). When chronically maintained, LFS seem to keep providing a benefit on speech, though often requiring therapeutic adjustment due to tremor or motor fluctuations reappearance.

We included PD patients with different levels of speech impairment in order to verify if lower frequency of stimulation could be a feasible option in the management of both DBS-treated patients with severe and mild dysarthria. Indeed, since also mild dysarthria can affect patient's perceived QoL, we aimed to verify if fine-tuning of stimulation parameters could be attempted also among these patients without losing an optimal control of motor symptoms. In this case, acute switching to LFS gave a statistically significant though not subjectively meaningful improvement of speech intelligibility. It has been suggested that an apparent improvement of axial signs with LFS is likely to appear only among patients who have a detrimental effect with HFS.[210] In agreement with this hypothesis, we found a more evident and clinically meaningful

benefit of LFS at follow-up among patients with severe speech impairment who presented a detrimental effect of HFS on speech. On the contrary, patients with mild speech impairment are not likely to benefit from LFS. As the volume of activated tissue depends on stimulation voltage, it has been suggested that LFS and high voltage can activate some critical mesencephalic structures, especially the mesencephalic locomotor area and the fasciculus cerebellothalamicus, that are conversely inhibited by chronic HFS resulting in dysarthria worsening. [211]Our findings confirm this hypothesis. However, it may happen that LFS does not maintain its effect on motor symptoms [212, 213]with consequent reappearance or worsening of motor fluctuations or tremor in few months, thus requiring stimulation or medication adjustment. The reason why such a benefit is not maintained over time remains to be clarified. Chronic HFS of the STN seems to cause long-term adaptation in the sensorimotor network, which results in reduced expression of subthalamic beta band oscillations and neural synchrony. [214]It would also be worth investigating if long-lasting LFS is related to phenomena of neuronal adaptation, in order to verify if cyclic stimulation frequency i.e. a nocturnal HFS and a daily LFS- could prevent the occurrence of long-term tolerance to LFS. Alternatively, if patients do not tolerate LFS over time, due to the worsening of motor symptoms that cannot be stabilized by medication adjustment the occasional and transient use of LFS could be considered, based on patients' needs.

As expected for advanced PD patients, L-dopa intake did not give an additional benefit on speech impairment. [179]At the same time, dyskinesias increment after L-dopa intake is probably not sufficient or not severe enough to influence respiratory control and consequently affect speech, as it could be expected for DBS patients who have an optimal motor control. [144]

The rate of L-dopa motor complications was higher among patients with severe speech impairment. However, among those patients, motor complications were more severe, though not significantly (data not shown), even before DBS and the motor effect of stimulation was significant in both groups. These data, along with the neuroimaging confirmation and the absence of stimulation-induced pyramidal side effects, support a correct position of the active contact.

The findings of our study are firstly limited by the lack of blinding for the neurological assessment, which was maintained only for SLT evaluations. Secondly, recordings were not performed in an acoustic laboratory setting. Nevertheless, the quality and reliability of the recordings were evaluated by a SLT. Moreover, it should be considered that a feasible, sensitive

and standardized tool for dysarthria assessment among PD patients has not been defined yet. Herein we adopt a brief and informative protocol for automatic acoustic assessment of DBS-treated PD patients. Further studies with larger sample should be performed to elaborate a standardized protocol for pre and post-surgical speech assessment of PD patients.

In conclusion, the acute switching to LFS seems to be a feasible option for STN-DBS patients with severe speech impairment at HFS. The possible application of alternative and new stimulation options that can widen the therapeutic window such as the use of short pulse width, directional leads or adaptive stimulations should also be investigated among DBS treated PD patients with severe speech impairment.

GENERAL DISCUSSION

Late-stage Parkinson's disease: further evidences for a new established clinical phenotype

In the last two decades, the definition of PD stages has much moved forward, with particular development for a better characterization of disease outers, i.e. the prodromal phase and the advanced one. [27, 28, 31]

The clinical spectrum of advanced PD patients has become larger and larger and the LSPD stage concept has emerged. [52] However LSPD can be still considered an orphan population, only partially or not at all included in RCTs and whose clinical management is still challenging. [64]

Our work brings a contribution in the definition of this recently recognized PD stage. We adopted in all our studies a previously suggested operational criteria for LSPD identification, [52] confirming that the combination of the HY, focused on motor and axial impairment, and S&E, focused on disability, could efficiently capture the wide spectrum of these highly disabled PD patients who could be partly missed using the only HY criteria (see CHAPTER 1). [48, 52] The assumption of a closely combination between dementia and postural instability can be rarely contradicted by the fact that PD patients may become demented before losing balance. Thus we strongly suggest the use of this operational criteria, [52] not previously adopted by other research groups, for future studies specifically directed to the latest disease stage.

PD has been defined as a clinical syndrome associated with a distinctive pathology.[3] Being a neurodegenerative disease PD evolves, displaying different clinical features throughout the disease course. [31] Interestingly in later disease stages, PD patients can present symptoms usually observed in atypical parkinsonism that all together depict a very peculiar clinical phenotype. We remark again the relative importance of L-dopa related MC, thus confirming that treatment for MC should be less of a priority. [52] At the same time, we reaffirm the predominance of dementia and autonomic symptoms, particularly urinary disturbances, which substantial impact patients' HR-QoL and caregiver distress (CHAPTER 1). [54, 56, 57]Sleep disturbances prevail among the most common NMS, showing a higher prevalence if compared to few previous reports, at least regarding daytime sleepiness that interest almost 90% of our patients. [54, 56, 57] However, among LSPD patients even the clinical identification of a wakefulness state can be challenging, as some of these patients spend several hours/day in a sort of apathetic state, with eyes closed, hardly discernible from sleepiness with no neurophysiological assessment. Joint

and skeletal deformities appear as a quite frequent disease complication (20%) and should be always investigated as cause of pain or ailment. The presence of swallowing problem can be defined as a red flag for disease severity and poor outcome (CHAPTER 1 and following paragraph). A further differentiation of LSPD from previous stages is supported also by a lower L-dopa responsiveness, particularly evident for axial motor symptoms (CHAPTER 1-2 and 4). Taken as a whole, the LSPD population appears as a distinct clinical phenotype, recognizable by definitive clinical criteria, with peculiar mild responsiveness or unresponsiveness to pharmacological treatment and a promising and growing impact on health care system. The number of age-dependent diseases, like PD, is high in high-income countries and increasing in low-income countries due to increasing with longer life expectancy and improving in medical care. [8, 215] Indeed, a systematic analysis published in 2017, has showed as burden of neurological disease, measured by the disability-adjusted life years (DALYs), i.e. the sum of years of life lost due to the disease and years lived with the disease, progressively increased from 1990 to 2015. [216] PD, particularly LSPD, is likely to substantially contribute to an increment of DALYs. An accurate definition of clinical needs and therapeutic management of LSPD patients is crucial for a functional allocation of health care resources in order to reduce the disease burden of this orphan population.

Markers of disease progression in late-stage Parkinson's disease

Markers of disease, intended as the starting of clinical manifestations of a neuropathological process, and markers of disease progression are of crucial importance in neurodegenerative diseases management, the first in view of the development of possible disease-modifying therapies and the second to monitor the disease evolution and eventually prevent its aggravation. [125] It is now available a rapidly growing list of proven biomarkers and clinical markers of prodromal PD as in the last decade research efforts have been focused on pre-clinical stages. [28, 31]

Throughout the disease course, few clinical markers can be considered as red flags in terms of disease progression. First of all, the appearance of troublesome MC, that classically defines the beginning of PD advanced stage and implies a definitive deterioration in patients' functional independence, [41, 42] widening the spectrum of treatment possibilities to possible device-aided therapies. [44, 217] Secondly, a further step towards PD end-stage is represented by the appearance

of one the main four disease milestones, i.e. frequent falls, visual hallucinations, cognitive disability and need for residential care, which all together can precede death of about five years.[52, 59] LSPD appears to be a good clinical model to identify the milestones that cause most disability and predict mortality, highlighting the symptoms that should be targeted for drug development at earlier stages of PD. So far, there were no data on how PD patients who have already reached the latest disease stage evolved and on which possible clinical indicators of poor prognosis clinicians should focus their attention. In terms of rate of clinical progression our work showed as LSPD patients differently evolve, even in this latest disease stage with an even faster rate of clinical progression, if compared to previous disease stages[37], at least regarding motor symptoms (CHAPTER 1). It seems that, regarding motor symptoms, PD present a certain rate of clinical progression in early stage (2.4 point of the UPDRS-III within the first 5 years)[38] that can slow down in advanced disease stages, [37] thought a faster recovery of disease progression is still possible at the very end of the disease. This finding is in line with the recently published paper of Ding and colleagues on a longitudinal assessment during a mean of 13.3 years of 34 PD patients enrolled before treatment initiation, that showed as motor deficit appeared to accelerate toward the end of the disease course in 27 patients who had died.[94] A progression is not present for L-dopa MC, which tend to decrease, maybe due to a roof effect, as those symptoms are likely to have already reached a peak during advanced stage and the neuropathological progression of the disease also contribute to an attenuation of this phenomena in the latest disease stage.[138, 218]

In terms of red flags for a poor outcome, the presence of swallowing problems seems to be the strongest clinical indicators, also sustained by the fact that pneumonia and food asphyxiation were listed among death causes (CHAPTER 1). Secondly, the presence of dementia that is strictly linked to severe dysphagia and the occurrence of death, along with the need for a formal caregiver and institutionalization in a nursing home are clinical and social markers for a poor prognosis. Interestingly, in spite of a suggested more benign and slowly progressive course among TD patients if compared to AK or PIGD ones, clinical phenotype has no more influence on disease progression, as observed in a long-term prospective study over about 18 years.[89] Among all those negative predictors, we have to highlight the role of a positive predictor, which is the presence of MC, particularly dyskinesias (CHAPTER 1 and 2). Indeed, as reported for previous disease stages, the presence of MC can be still related to patients' better functional ability as being correlated to a greater pharmacological treatment response. [58, 136] However, the relevance of this finding is partly mitigated by the fact that L-dopa responsiveness seems to

have a minimal impact on patients' prognosis, though probably still related to a higher patients QoL. Taken as a whole, we may argue that once reached the LS, PD patients with severe dysphagia, rapidly evolve to a very end-disease stage, independently from their L-dopa responsiveness.

In spite of the clinical markers being considered the most-established means for PD diagnosis and progression, several neuroimaging techniques, such as [^{123I}]β -CIT SPECT or [^{18}F]DOPA

PET, high resolution MRI-based nigrosome/neuromelanin assessment and transcranial ultrasound of the SN, have been adopted and proposed as biomarkers of nigrostriatal dopaminergic lesion and nigral degeneration progression. [126-128] Neuroimaging tools have been variably criticized as reliable biomarkers for PD progression due to several limitation in terms of cost, time consuming and variable or poor correlation with disease progression.[219, 220] NM-MRI study is not free of some of those limitations, especially regarding the long acquisition time and the heterogeneity of data on its correlation with disease progression that could be partly accounted to the lack of consensus acquisition and analysis protocol. We observed as a decrement in SN-NM area goes with disease progression, being able to differentiate *de novo* from LSPD patients, though the number of patients or technique accuracy were not enough to distinguish LSPD from an intermediate stage (CHAPTER 1). So far, we cannot still affirm that LSPD patients are clearly discernible from advanced/intermediate PD stages by means of NM-sensitive MRI studies.

Levodopa in later Parkinson's disease stages

LSPD patients are highly dependent on caregivers for ADL, owing to treatment-resistant motor symptoms or NMS.[52-54] At the same time, due to the frequent occurrence of AEs - namely, psychosis and excessive daytime sleepiness - induced by antiparkinsonian drugs, a regimen simplification of treatment strategy, based on the unique use of L-dopa as antiparkinsonian therapy and drugs for psychosis, dementia and psychiatric symptoms such as depression, apathy and anxiety, has been recommended. [52, 54] In this context, a clarification of the role and effect of L-dopa among LSPD is crucial. Our work specifically investigated this aspect, analysing the "multimodal" L-dopa effect on motor symptoms, NMS and finally focusing on speech, as one specific troublesome NMS of later PD stages (CHAPTER 2, 3,4, and 5, respectively).

The magnitude of L-dopa response to a supramaximal dose varies from 11% to 18% of the MDS-UPDRS-III, that correspond to 8.5 and 12.7 points of the scale, respectively. The clinical significance of this motor response resulted marginal according to the CGI-I and the change in the S&E between OFF and ON state, though more evident for some appendicular signs, especially tremor and rigidity and, partially, on gait. Regarding motor symptoms, our results seem to delineate a clinical profile of LSPD patients who are more likely to respond to L-dopa, and may benefit from a cautious dose increment – namely for TD patients, with no dementia and who still present MCs. Nevertheless, when managing LSPD patients' treatment, clinicians should be always keep attention to not alter the frail and unsure balance between a mild motor benefit, unresponsive symptoms and treatment-related AEs. This is the reason why an eventual dose increment should be "cautious". Indeed, one-fourth of our patients developed OH, 22% drowsiness and no L-dopa effect was observed on pain, anxiety, fatigue and speech. Reasons for unresponsiveness can be the partial involvement of the dopaminergic pathways in non-motor or axial symptoms etiopathogenesis,[5, 155, 159 221, 222] the high frequency of AEs and the mild motor response which was conversely higher among advanced patients who had a significant response of anxiety and pain. Whatever the cause, L-dopa does not represent a therapeutic option for LSPD patients who suffer from severe dysarthria, anxiety, fatigue and pain and particularly for this last complain clinicians should look for joint and skeletal deformities, other than MC-related pain causes.

Our finding on a mild acute response should not be translated in an L-dopa ineffectiveness nor in recommendations for drug suspension, neither for dose decrement in absence of AEs. The L-dopa "long-duration response", which does not seem to follow the drug's plasma concentration and can persist for hours to days after the drug has been stopped[22], should be taken into account, even in this latest stage. Finally, in spite of these recognized limitations, we propose to consider the L-dopa acute challenge test as a reliable tool for treatment responsiveness monitoring even among LSPD patients, as it happens in early disease stage [223, 224] or for device-aided therapies patients' selection. [217, 225]

Device-aided therapies in later Parkinson's disease stages: a new scenario

Even if there is no accurate and recognized valuation, about 5% to 10% of PD patients are eligible for DBS [226] and the percentage of patient eligible for one of the three available device-aided therapies, namely DBS, LCIG and CAI, is likely to be around 10%-15%. The introduction of device-aided therapies has definitively improved PD patients' Qol and functional independence[227-230], though not preventing the emergence of other sources of disability.[231] Indeed, so far no strong evidence support a neuroprotective role of those invasive treatments. Even if the disease course has been not changed and PD patients could eventually enter in a LS of the disease, independently from the invasive treatment to which they underwent, [52]device-aided therapies have widen the spectrum of treatment possibilities for MC and slightly changed the management of poor L-dopa responsive symptoms, at least in advanced disease stage. Our study on LFS effect in dysarthric PD patients submitted to STN-DBS (CHAPTER 5) offers a good example of this scenario. Speech disorders remain a poor L-dopa responsive condition [172] and, as a rule, device-aided therapies have an effect only on L-dopa responsive symptoms. [74, 217] Thus STN-DBS is not likely to offer a benefit on dysarthria related to PD disease progression. However the approach to speech disturbances in a DBS-treated PD patient should sift through several treatment possibilities, based on the assessment of chronic or acute stimulation-related effect or AEs. Indeed, the fine-tuning of stimulation parameters has shown to be a possible therapeutic option for a sub-group of patients with severe dysarthria during chronic standard HFS (CHAPTER 5), even if L-dopa showed no effect on speech among those patients, at least in combination with stimulation. Moreover, the spectrum of possible fine stimulation adjustments will expand in the next few years due to new recent stimulation options, such as directional leads or novel pulse parameters.[232] At the same time, chronic LCIG treatment has been shown to have a beneficial effect on some NMS, with the exception of urinary disturbances[233] and on FOG at least up to 1 or 2 years of treatment[234, 235], probably due to a more constant dopaminergic drug delivery associated with fewer response fluctuations than oral L-dopa. CAI seems also to have an overall beneficial effect on NMS of PD patients, including neuropsychiatric symptoms, sleep disturbances, pain, urinary dysfunction, and impulse control disorders[236], and its possible and cautious use in elderly PD patients with cognitive impairment have been recently suggested. [237] We are aware that if a reduced NMS burden under device-aided therapies treatment occurred, it is likely alongside a sustained improvement in motor symptoms and "OFF" time. Moreover this improvement is more likely

to occur in advanced PD patients than is LSPD ones. Nevertheless, two points should be highlighted: i) patients under device-aided therapy treatment may benefit of a wide spectrum of fine adjustments, even for the most challenging parkinsonian symptoms; ii) a sub-group of advanced PD patients previously submitted to device-aided therapies, will enter the LS disease raising new challenging questions on how invasive treatment should be managed in the latest disease stage, how they interact with oral treatment and when and how they should be interrupted (see next paragraph).

Implications for clinical practice

LSPD represents a recently identified clinical stage of PD, clearly discernible from the advanced one in terms of clinical features, therapeutic response to L-dopa, AEs frequency and susceptibility, functional independence in ADLs, prognosis and caregiver burdens.

Health care professionals should actively investigate, with the help of caregivers, the presence of the most troublesome symptoms for these disabled patients, such as falls, hallucinations, coking, cognitive decline, sleep and urinary disturbances. Among those symptoms, swallowing problems should receive a particular attention and a prompt assessment by a phoniatrician should be considered as soon as the first symptoms appear or regularly in the latest disease stage.

L-dopa treatment, as monotherapy, remains the main option in terms of anti-parkinsonian medication. Not PDD patients, who still present MC and who complain of tremor and rigidity, may benefit from a cautious L-dopa dose increment. Indeed, in LSPD, MC are not an additional source of disability but an indicator of better L-dopa responsiveness. At the same time, attention should be done for possible drowsiness or OH appearance and clinicians should be aware on the L-dopa inefficacy on NMS and on axial symptoms, being speech the most unresponsive axial symptoms and gait the one that may rarely still respond. At the same time, in the management of severe dysarthria in advanced PD patients treated with STN-DBS, should consider the possible detrimental effect of chronic HFS and a possible benefit reached by means of fine stimulation parameters adjustment.

Overall, due to a complex clinical picture, LSPD patients and their familiars should be treated by means of a multidisciplinary holistic approach, which include both pharmacological and non-pharmachological treatments, such as phoniatric and physical rehabilitation interventions, social and psychological support. [70, 72, 238] Finally, an in-home based-care should be definitively considered. Almost all our patients were visited at home and the present institution-based medical approach has too many shortcoming for LSPD patients, being based on once or twice a year in-hospital visits that often do not reflect the daily patient situation, implies time, costs and consuming patients dislocations for those highly disabled patients. [239, 240]

Implications for research

LSPD patients care is a long list of unmet clinical needs that reflect into many research implications.

Neuroanatomical and neuropharmacological bases of non-motor abnormalities in PD remain largely undefined and basic research should be focused on the pathogenesis and neuropathology of L-dopa-unresponsive symptoms, which represent the major cause of disability of LSPD patients. Moreover, non-dopaminergic drugs may improve the tolerability profile of antiparkinsonian agents avoiding the classical dopaminergic AEs and could be a good therapeutic option in later disease stages.

Overall, an effort should be made in order to include LSPD patients in RCTs, especially for those studies that aims to investigate novel non-dopaminergic drugs or innovative care approaches. Indeed, so far very few clinical trials had specifically included LSPD patients. Regarding an innovative care approach, cost-effective and feasibility studies should be principally focused on a multidisciplinary/palliative in-home based care, in order to verify if this approach could at least be comparable and hopefully superior to an institution-based approach both in terms of costs and patients/caregivers' Qol. Therapeutic interventions on LSPD patients' "environment", particularly on caregivers and home should be also further investigated.

Most of the instruments available to assess LSPD patients seem to be partially adequate or mostly inadequate, above all for NMS, probably because clinometric properties of those scales have not specifically tested among LSPD patients who are usually hardly testable due to the presence of dementia, behavioural disorders and severe dysarthria. The current assessment tools should be validated and eventually adapted to LSPD patients. Equally, also non-pharmacological interventions, particularly swallowing training, should be adapted to LSPD patients.

Clinical markers can identify LSPD and few clinical indicators of poor outcome or poor treatment response have been found. At the same time, it could be useful to identify reliable biomarkers for advanced PD patients who are likely to briefly enter in the latest disease stage or LSPD patients who are likely to respond to specific pharmacological treatment or not.

Finally, guidelines and recommendations on the management of LSPD patients under device-aided therapies, including consideration on treatment interruption, should be elaborated. Indeed, a sub-group of PD patients previously submitted to invasive treatment will reach the LS requiring a high level of specialization in movement disorders treatment management. Device-aided therapies can be finely tuned in order to widen the therapeutic options, but they can also couple with several complications especially among elderly PD patients with long disease duration.

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Research Report

Substantia Nigra Neuromelanin as an Imaging Biomarker of Disease Progression in Parkinson's Disease

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Abstract.
Background: A specific T1-weighted magnetic resonance imaging (MRI) sequence has been shown to detect substantia nigra (SN) neuromelanin (NM) signal changes that accurately discriminate Parkinson's disease (PD) patients from controls, even in early disease stages. However, it is unclear what happens to these SN changes in later disease stages and if they can be a marker of disease progression.
Objective: to investigate the pattern of SN-NM area loss and contrast ratio (CR) intensity changes in late-stage PD (LSPD) compared to earlier disease stages.
Methods: A comparative cross-sectional study was performed, analyzing SN-NM MRI signal in LSPD (Schwab and England Activities of Daily Living Scale score <50 or Hoehn Yahr Stage [HY] >3), comparing this group with *de novo*, 2–5 year PD and controls. SN-NM signal area and CR values for the internal and lateral SN regions were obtained with semi-automated methods.

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Results: 13 LSPD, 12 *de novo* patients with PD, 10 PD patients with a 2–5 year disease duration, and 10 controls were included. NM signal area was significantly decreased in LSPD compared to *de novo* PD (*P-value* = 0.005; sensitivity: 75%; specificity 92% and AUC: 0.86). In the lateral SN region, a decrease in the CR was detected in all PD groups compared to controls; despite not reaching statistical significance, a slight increment was observed comparing LSPD to 2–5 year PD. NM signal area significantly correlated with HY (R = –0.37; $P < 0.05$) and Movement disorder Society Unified Parkinson's Disease Rating Scale part II (MDS-UPDRS) (R = –0.4; $P < 0.05$) while a weak correlation was found with MDS-UPDRS part III (R = –0.26; *P*: 0.1).

Conclusion: SN area evaluated by NM-sensitive MRI may be a promising biomarker of nigral degeneration and disease progression in PD patients.

Keywords: Neuromelanin, Parkinson's disease, late-stage, disease progression, biomarker

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a selective loss of pigmented neurons in the substantia nigra (SN) *pars compacta* (SNc) and *locus coeruleus* (LC) and by the appearance of Lewy bodies [1, 2]. Approximately 60–70% of dopaminergic neurons of the SNc are lost before the onset of clinical PD symptoms and their degeneration progresses throughout the disease [3].

The degree of neuronal loss in the SNc is correlated to PD severity, which confirms the potential of SNc imaging for tracking disease progression [4].

The pronounced depigmentation of SNc neurons is related to the loss of neuromelanin (NM), which, in PD patients, occurs in the whole *pars compacta* region though preferentially affecting the ventrolateral part [5]. Over the last 10 years, new T1-weighted magnetic resonance imaging (MRI) sequences have been shown to detect a significant reduction in the SN-NM signal in PD compared to healthy subjects; these sequences also enable the differential diagnosis with essential tremor [6]. Furthermore, a reduction of SN and LC contrast ratios (CR) has been reported in PD patients distinct from atypical parkinsonian syndromes [6–11]. These NM changes have a high diagnostic sensitivity and specificity for PD diagnosis, even in early clinical stages [8, 12–14].

However, the relative ability of NM-sensitive MRI to mark disease progression and to detect potential differences in pathophysiological processes still remains unclear. Currently, very few studies have looked at longitudinal changes in the SN NM with MRI; inconsistent results have been reported, that could be related to differences in MR acquisition parameters and data analysis [12, 15–17]. Likewise, only a few studies have suggested a potential correlation of NM SNpc signal intensity loss (or CR) or NM-volume loss with disease severity, i.e. Hoehn and Yahr rating scale (HY) or Unified Parkinson's Disease Rating Scale (UPDRS) scores [8, 11, 14, 16].

The purpose of this study was to investigate the pattern of SN-NM area loss and CR intensity changes in late-stage PD (LSPD) patients, compared to de novo PD patients and PD patients with a 2–5 year disease duration, and thereby evaluate NM changes throughout disease progression.

PATIENTS AND METHODS

Patients

We performed a comparative cross-sectional study that included 45 subjects: 13 LSPD, 12 *de novo* PD patients, 10 PD patients with a 2–5 year disease duration, and 10 healthy subjects.

Inclusion criteria for healthy subjects, *de novo* PD patients and patients with a 2–5 year disease duration has already been reported in a previous paper [12]. Patients were recruited from the Movement Disorders Unit of the University Hospital of Santa Maria, Lisbon. PD was defined according to the UK Brain Bank criteria [18] and diagnosis was made by a movement disorders specialist. LSPD was defined as PD patients with either a Schwab and England score (S&E) < 50 (MED ON) or a Hoehn &Yahr stage (HY) >3 (MED ON) [19, 20].

PD patients were rated using the UPDRS, except for the LSPD group who were evaluated by means of the Movement Disorder Society (MDS) UPDRS [21], while MED ON. Conversion from the UPDRS-part II and UPDRS-part III to the MDS-UPDRS part II and MDS-UPDRS part III respectively, was performed adopting the algorithm proposed by Goetz and colleagues [22]. *De novo* PD patients were not on antiparkinsonian medication and they were all <6 months since the beginning of clinical symptoms. L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversions [23].

The Local Ethical Committee approved the study and all patients provided informed consent.

Imaging protocol

A 3.0 T Phillips scanner (Phillips Achieva; Phillips Medical Systems, Best, Netherlands) was used to acquire all data. A T1 -weighted fast spin echo NM-sensitive pulse sequence was used as previously described by Sasaki and colleagues, [24] with a repetition time/effective echo time of 633/10 ms, echo train length of 3, 20 slices with 2.5 mm of thickness and intersection gaps of 0 mm, field of view of 220 mm, matrix size of 548 × 474 (pixel size of 0.40 × 0.40 mm^2) and an acquisition time of 8 min. Slices were set in an oblique axial plane perpendicular to the fourth ventricle floor and covering from the posterior commissure to the inferior border of the pons. Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) images were also acquired for volumetric analysis, with 0.74 × 0.74 × 1.0 mm^3 resolution, TR/TE of 9.6/4.6 ms. In case of motion artefact, the sequence was repeated adjusting the slice positioning and reiterating to the patient on the importance of remaining still.

Image analysis

The software OsiriX (OsiriX Lite version 8.0, Pixmeo, Geneva, Switzerland) was used to perform image analysis. A Gaussian filter (full width at half maximum of 0.8 mm) was applied to reduce image noise, prior to performing image segmentation using the confidence region growing algorithm. As the high signal intensity SN was always visible in three slices, the middle slice, corresponding to the greatest SN volume was selected for segmentation.

Two symmetrical seed points were manually defined on the most medial part of the high intensity area in the SN, and as close as possible to an imaginary straight line passing through the bottom of the interpeduncular cistern. The SN CR were assessed by positioning circular regions of interest (ROI), covering approximately 26 pixels, in the internal and lateral parts of both sides of the SN and in the lateral part of the *crus cerebri*, taken as a reference. The CR were calculated using the following equations:

$$CR_{iR} = \frac{SN_{iR}}{CC_R}$$

$$CR_{iL} = \frac{SN_{iL}}{CC_L}$$

$$CR_{lR} = \frac{SN_{lR}}{CC_R}$$

$$CR_{lL} = \frac{SN_{ll}}{CC_L}$$

Where $CR_{iR,iL,lR,iL}$ correspond to the CR of the internal right (iR), internal left (iL), lateral right (lR) and lateral left (lL) regions of the SN, respectively. $SN_{iR,iL,lR,lL}$ are the average values of the signal intensities within the ROIs positioned on the described regions of the SN, and CC_R, L the average values of the signal intensities within the ROIs positioned on the right and left region of the *crus cerebri*, respectively (Fig. 1).

The midbrain and brainstem volumes were estimated using Freesurfer® for the automatic segmentation of the MPRAGE images. To account for inter-subject variability, the fraction of midbrain to brainstem volume (MBF) was calculated.

Statistical analysis

The Wilcoxon Ranked Test was used to test statistical differences between right and left NM area among subjects of each group. Kruskal-Wallis tests were employed with P-values corrected for multiple comparisons using the Bonferroni method. Potential differences in the SN areas and in the clinical characteristics among the different groups were evaluated.

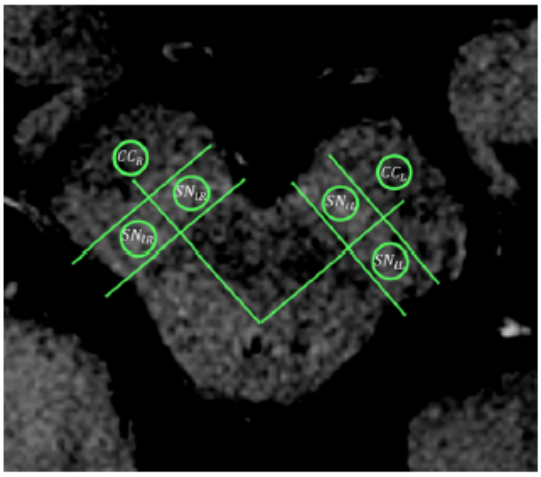

Fig. 1. Representative CR assessment by means of circular regions of interest on an NM-sensitive T1-weighted MRI. CC_r: crus cerebri right; CC_L: crus cerebri left; SN_{iL}: substantia nigra, left internal region; SN_{lL}: substantia nigra, left lateral region; SN_{iR}: substantia nigra, right internal region; SN_{lR}: substantia nigra, right lateral region.

The Wilcoxon signed-ranked test was performed to evaluate differences between the area and CR of both sides of the SN of each subject.

Receiver operating characteristic curve (ROC) analyses were performed to determine the sensitivity, specificity, cut-off optimal values and the area under the curve (AUC) for distinguishing between the different PD groups. The Pearson product-moment correlation coefficient was used to evaluate the dependence between the MDS-UPDRS Part III score, MDS-UPDRS part II, LEDD, HY stage, age and the mean area of the SN and CR*l*/CR*i* results. Also, the dependence between the MBF and the SN areas was evaluated.

Differences in the clinical characteristics were also assessed. The chi-squared test was performed to evaluate differences in the sex distribution among groups. For comparison of the age between groups as well as for the MDS-UPDRS total score and MDS-UPDRS Part III, the Kruskal-Wallis test was used. A P value of 0.05 was considered significant.

All analyses were performed with the R software (Version 3.3.1, The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

MRI was performed on all subjects, and the image quality allowed a clear identification of the high signal area in the SN region as well as a semi-automatic analysis of all NM-MRI images.

The demographic and clinical characteristics of all subjects are detailed in Table 1. LSPD patients had a median disease duration of 14 years [IQR: 9–17]. They were significantly older compared to controls and *de novo* PD patients and had a worse HY stage and MDS-UPDRS part II compared to the *de novo* and 2–5 year PD groups. MDS-UPDRS part III scores of LSPD patients were worse compared to the *de novo* and 2–5 year PD groups, but the difference was statistically significant only for 2–5 year PD patients (Table 1).

We found no difference between the left and right NM areas ($0.31 < P < 0.79$) and so the mean right/left area value was used in all subsequent analysis.

The median SN-NM area obtained for *de novo* PD patients, 2–5 year PD, LSPD groups and healthy subjects is detailed in Table 1.

The median SN-NM area was markedly decreased in PD groups compared to controls (Fig. 2) with a P value of 0.002 for *de novo* PD patients and a

Table 1

Demographic, clinical and neuromelanin assessment data of patients and controls. Values are presented as median [IQR: 25th–75th percentile] if not otherwise specified. NA, not available; LEDD, levodopa equivalent daily dose; CR, contrast ration; HY, Hoehn and Yahr rating scale; MDS-UPDRS, Movement disorders society Unified Parkinson's disease Rating Scale Comparisons: a) controls versus *de novo PD*; b) controls versus 2–5 years PD; c) controls versus LSPD; d) *de novo* PD versus LSPD; e) 2–5 years versus LSPD; f: *de novo* PD versus 2–5 years PD. Statistical significant results are in bold characters

	Healthy subjects	*De novo* PD	2–5 year PD	LSPD	*P value*
Number (female/male)	10 (4/6)	12 (7/5)	10 (2/8)	13 (7/6)	0.3
Age, y	60 [55–69.2]	62.5 [52.5–73.7]	66 [63.5–71.2]	78 [68.5–81.5]	a, f: 1; b: 0.8; **c: 0.001; d: 0.003;** e: 0.08
HY	NA	2	2	4	**d - e: <0.001**
LEDD	NA	0	480 [325–810]	1040 [725–1325]	**e <0.01**
MDS-UPDRS part II	NA	6.2 [3.5–10.6]	10.1 [1.7–12.8]	36 [30–40.5]	**d-e: <0.001;** f: 0.1
MDS-UPDRS part III	NA	32.3 [28.7–47]	24.5 [13.4–43.1]	51 [41–53.5]	f: 1; **e: 0.02;** d: 0.09;
Area (mm^2)	40.63 [33.03–55.64]	27.7 [17.13–360.4]	22.65 [8.64–46.84]	18.68 [12.50–26.47]	**a: 0.002; b, c <0.001; d: 0.005;** e: 1; f: 0.8
CR Internal region	1.16 [1.11–1.19]	1.15 [1.09–1.21]	1.12 [1.05–1.16]	1.12 [1.09–1.18]	0.06
CR Lateral region	1.10 [1.02–1.12]	1.06 [0.10–1.13]	1.03 [0.99–1.08]	1.04 [0.10–1.1]	**b: 0.008;** a,c:0.1; d,e,f: 1

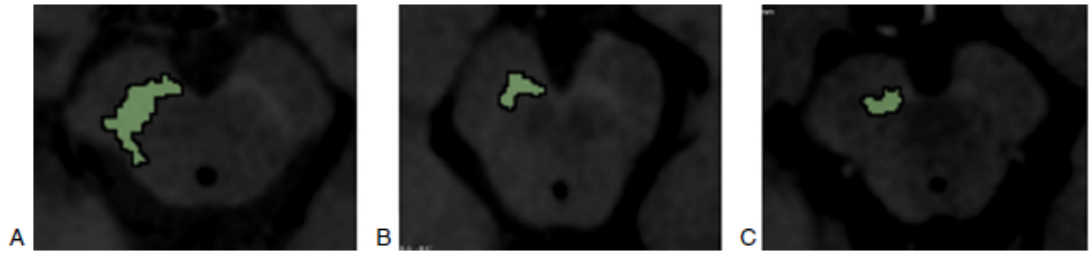

Fig. 2. Neuromelanin (NM) area selection on NM-sensitive MRI of the SN of a healthy control (A), a de novo PD patient (B) and a LSPD (C) patient.

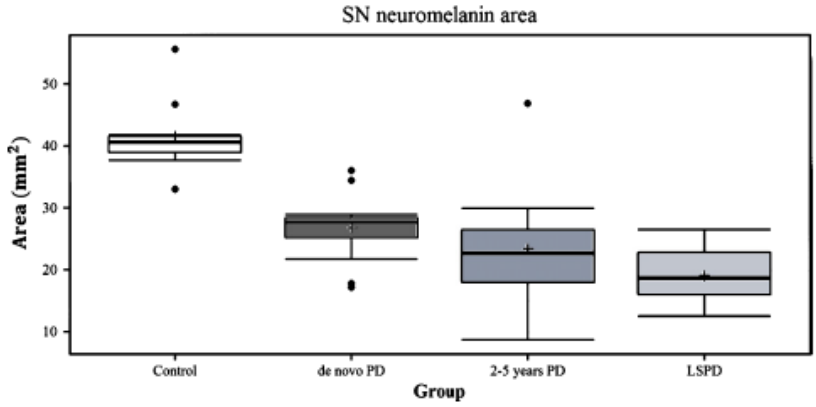

Fig. 3. Median area values of the SN high intensity region on NM-sensitive MRI in *de novo PD* patients, 2–5 year PD patients, LSPD patients and controls.

P value <0.001 for 2–5 year PD and LSPD groups (Table 1). The NM area of the LSPD group was significantly smaller when compared with the *de novo* group ($P = 0.005$) but not when compared to the 2–5 year PD group (Table 1 and Fig. 3).

On ROC analyses, the sensitivity and specificity of the SN high signal area for discriminating the LSPD group from earlier PD groups were: a) 75% and 92%, respectively, with a cut-off value for the area set at 26.31 mm^2 and an AUC of 0.86 if compared to *de novo* PD (Fig. 3, Panel B); b) 70% and 62%, respectively, with a cut-off value for the area set at 19.29 mm^2 and an AUC of 0.65 if compared to 2–5 year PD; (Fig. 4, Panel C). The sensitivity and specificity for discriminating the 2–5 year PD group from the *de novo* group were 67% and 80%, respectively, with an area cut-off value of 27.16 mm^2 and an AUC of 0.69 (Fig. 3, Panel A). Finally the sensitivity and specificity for discriminating all PD patients from controls were 100% and 91%, respectively, with an area cut-off value of 33.02 mm^2 and an AUC of 0.969 (Fig. 4, Panel A).

No differences were found among right versus left CR in both medial and lateral SN across all groups, except for the LSPD group ($P < 0.05$). Thus, CR analysis was performed independently for left and right values. CR analysis for both right and left sides of the internal SN region showed no differences across all PD groups and controls. Concerning the lateral SN region, CR analysis showed a significant difference only for the left side between 2–5 year PD patients and controls ($P < 0.05$).

The median left and right CR results obtained for the internal and lateral SN region are detailed in Table 1. Across all groups no differences were found for the internal SN region ($P = 0.06$), while CR in the lateral region was significantly different between controls and 2–5 year PD patients ($P = 0.008$) (Fig. 5). Although no other statistically significant differences were found, a tendency for CR decrease was observed with disease progression for early-intermediate stage groups (Fig. 5). Contrary to this trend, an increment in CR was observed for the LSPD group if compared to the 2–5 year PD group (Fig. 4).

No statistically significant differences were found for the MBF across all groups (global *P*: 0.2) and no correlation was found between MBF and SN-NM area ($R = 0.14$; $P = 0.37$).

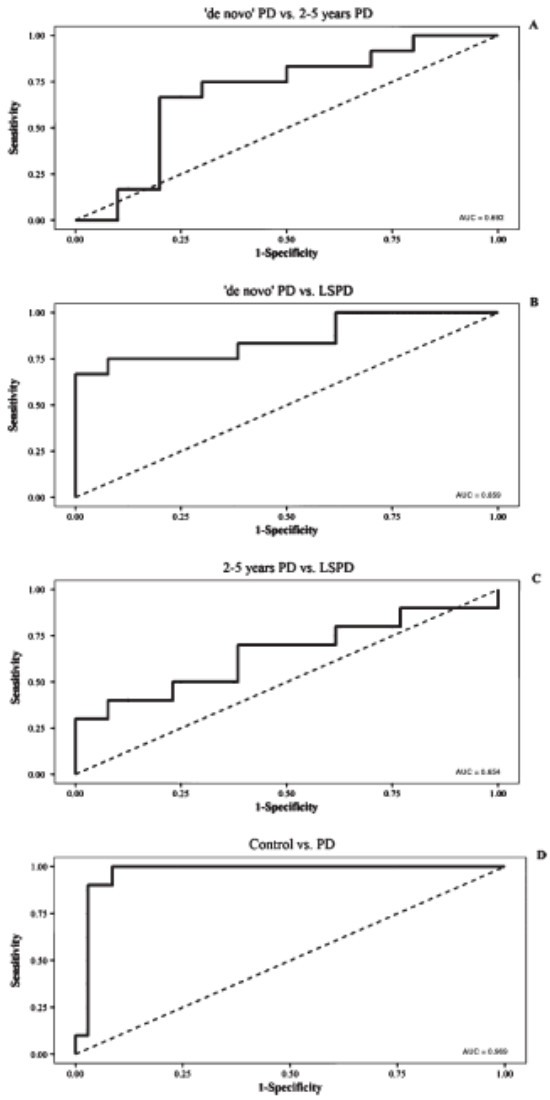

Fig. 4. Receiver Operator Characteristics (ROC) curves of the NM area for: a) differentiating between *de novo* PD versus 2–5 year PD patients (A); b) *de novo* PD versus LSPD patients (B); c) 2–5 year PD versus LSPD patients (C); d) PD versus controls.

No significant correlation was detected between SN-MN mean area and CR of the internal region (CR*i*) (R = 0.33; $P = 0.054$) and the CR of the lateral region (CR*l*) (R = 0.3; $P = 0.08$).

Considering all PD groups, MDS-UPDRS part III showed no correlation with SN-NM area (R = –0.26; *P*: 0.1). Negative moderate correlations were found between the SN-NM area and the MDS-UPDRS part II (R = –0.4; $P < 0.05$), LEDD (R = – 0.45; $P < 0.05$) and HY (R = –0.37; $P < 0.05$). No correlation was found between age and NM area values.

A moderate correlation was found between age and CR*l* (R = – 0.42; $P < 0.05$) and CR*i* (R = –0.36; $P < 0.05$). No correlations were found between HY, MDS-UPDRS part II, MDS-UPDRS part III, LEDD and CR*l* or CR*i*.

DISCUSSION

In the present study, we were able to identify a significant reduction in the NM-SN area compared to controls among several groups of PD patients belonging to different disease stages, i.e. from a very early stage up to LSPD. This is consistent with a tendency for NM depletion with disease progression.

Our results also confirm the ability of NM-MRI related measures for differentiating PD patients from healthy controls with high accuracy, even in the early disease stages, as reported in previous studies [8, 12, 13, 17].

The main objective of our study was to investigate NM-MRI alterations in an LSPD sample, to see the NM changes with disease progression and its potential as a biomarker of disease progression in PD. The NM-SN area presented a tendency to decrease with progressive disease stages, with statistical differences between *de novo* PD and LSPD patients. Furthermore, setting a cut-off value at 26.31 mm^2, we found excellent sensitivity, specificity, and AUC values for differentiating *de novo* PD and LSPD patients (75%, 92% and 0.86, respectively). There are very few studies that have explored NM-area modifications in PD evaluating early, intermediate and advanced PD stages (from HY stage 1 to 4) and all included small sample sizes. These studies reported conflicting results, although the use of different imaging and analysis protocols may partly account for these differences [12, 15, 16, 25]. Indeed, in a previous report we found no differences in SN area or length when comparing *de novo* PD with 2–5 year PD patients [12]. A few other reports suggest a tendency for SN-NM area reduction with disease progression: Schwarz and colleagues observed a tendency for a decrease in NM area when comparing six PD patients with HY stages 1–1.5 with four PD patients with HY stages 2-3 [15]. While Aquino and colleagues observed differences in NM area between twenty-two 3–5 year PD and twenty 6–10 year PD patients (HY stage <3) [25]. Finally, a recent study by Matsuura and colleagues reported longitudinal changes in NM-SN area in a group of fourteen PD patients, suggesting a decline of approximately

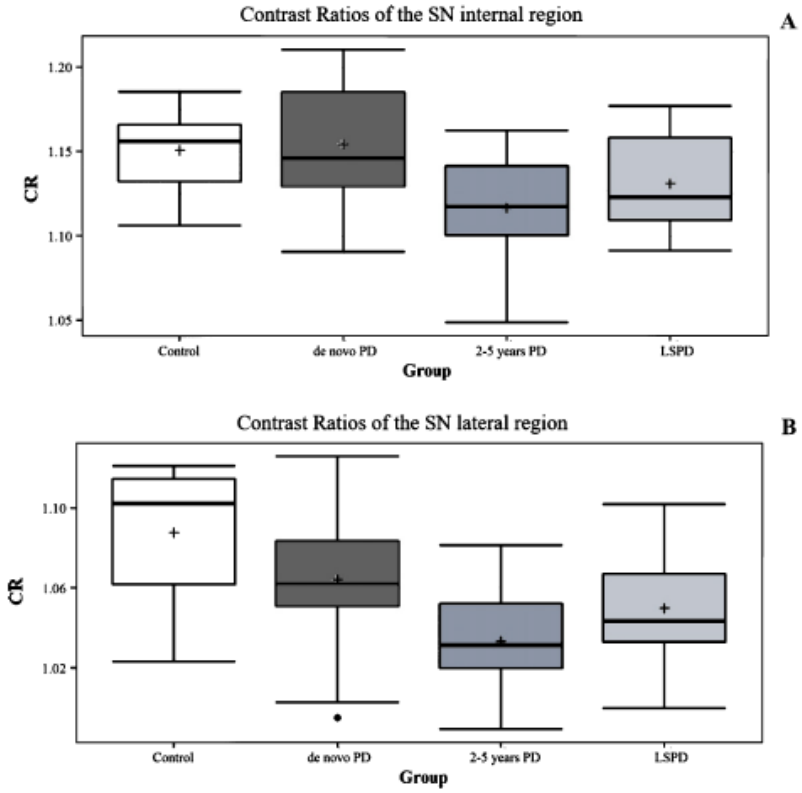

Fig. 5. CR values in in *de novo PD* patients, 2–5 year PD patients, LSPD patients and controls for the SN internal region (A) and lateral region (B).

17.5%, after one year follow-up, concomitant with an aggravation of HY stage (from a range of 1–3 to 2–4) [16]. However, to the best of our knowledge, this is the first study in which SN-NM area is specifically examined in a population of LSPD patients. Our findings are in agreement with the report of apparent disease stage- and duration-dependent volume loss of the SN-NM-sensitive region as reported in a manual NM volume analysis, performed on PD patients presenting HY stages 1 to 5 [8]. An age-related bias on NM area reduction among our sample of LSPD patients cannot be excluded, as those patients were statistically older when compared to *de novo* PD ones. However, a correlation with age was found only for CR values and not for NM area values. In the current literature there is no consensus on the loss of pigmented neurons during normal aging [26–28]. Nevertheless, throughout a sensitive and specific biochemical quantification of NM, we know that in the SNc this pigment linearly increases with age from the 10th year up to the ninth decade of life. [26, 29]. Moreover in normal ageing the fallout of pigmented neurons has a very low rate, i.e. 4.7% per decade [3]. Taken as a whole, our findings on NM area reduction among LSPD patients do not seem to be significantly influenced by age and are more likely accounted for by a stage-dependent modification as opposed to an age-dependent factor.

Though the MDS-UPDRS part III score showed no significant correlation with SN area depletion, we found a negative significant correlation of SN area with other indicators of disease severity, i.e. MDS-UPDRS part II and HY. Such a correlation is in agreement with our finding of NM area stage-dependent depletion, as suggested in a few other studies [14, 17]. The absence of a significant correlation between MDS-UPDRS part III and SN area depletion can be accounted for by the relatively high MDS-UPDRS-III scores of our *de novo* PD sample, probably linked to the medication-free condition of those patients and with the high frequency of tremor dominant type (11 over 12) [6]. Moreover, as showed in previous studies, the activities of daily life subscore, i.e. the MDS-UPDRS part II, may be a better biomarker of disease progression than other MDS-UPDRS sections [30–32].

To evaluate the possible impact of a midbrain volume reduction in PD patients which could have influenced NM measurements, the MBF was calculated for each group. As expected, the midbrain volume was similar between the groups and the calculated MBF showed no correlation with NM area depletion, confirming that individual midbrain volume does not explain the reduction of NM in PD [12].

Concerning the CR assessment, although a statistically significant difference was observed when comparing PD patients to controls, and a there was a tendency for CR decrease with disease progression, a small and non-statistically significant increment in CR was observed for the LSPD group compared to the 2–5 year PD group. Even if LSPD patients had a clearly worse clinical condition and longer disease duration when compared to 2–5 year PD patients, they were taking a significantly higher levodopa dose. Dopamine and dopamine agonists in standard dosages do not markedly affect DaT binding. A recent study found a correlation of the CR of the SNc and LC with DAT binding values [33]. Interaction between NM-SN signal and dopaminergic therapy is currently unknown but its influence cannot be excluded.

The pattern of pigmented neuron loss of the SN follows an opposite trend comparing PD patients with normal ageing to that observed for CR, with a greatest neuronal loss in PD (45% loss in the first decade), principally affecting the ventro-lateral part of the SN which is relatively spared in controls [3]. Accordingly, comparing healthy subjects with PD patients, we found a significant reduction of CR only in the lateral SN part. Those data suggest that CR*l* could be more appropriate than CR*i* in differentiating PD patients from healthy subjects. A few other studies on NM-CR in PD patients have reported heterogeneous results. Indeed, Ohtsuka and colleagues reported a NM-CR diminishing in the lateral-central part of SNc and LC in early (HY stage 1-2) and advanced (HY stage 3–5, during MED OFF) PD patients, compared to controls, but equally observed no difference between early and advanced patients, which is consistent to results from Schwartz and colleagues [15], however, no LEDDs were reported in either paper [17]. Conversely, Matsuura and colleagues reported a CR reduction during one-year follow-up observation with a correlation between CR values and disease duration, in spite of a LEDD increasing from about 380 mg to 630 mg [16]. Moreover, CR values did not show a significant correlation with indicators of disease severity (HY), further confirming that its alterations are not clearly coupled with disease progression [16] thereby suggesting that other confounding factors should be identified. Myoshi and colleagues found a stage-dependent CR reduction in the medial part of SNc, comparing 1-2 HY PD patients with 3–5 HY ones [34]. Taken as a whole, even if CR of SNc should give a measure of the density of melanized neurons, its relationship with disease progression in PD remains to be clarified. Finally a greater signal attenuation on NM imaging has been found in the LC when compared to SNc among PD patients [7, 17], though no difference between early and advanced PD patients were found even in the CR of the LC [7, 17].

A potential source of signal variability is the inhomogeneity in the B1 field, particularly relevant at 3.0T, which is known to affect image contrast. This effect should be accounted for in future studies, performing bias field correction prior to CR evaluation [35]. Future work should include assessing the variability in measured signal intensity and estimated NM-area associated to the acquisition and segmentation procedures. To assess the former, the acquisition procedure should be repeated after patient repositioning.

Several neuroimaging techniques, such as [18F]fluorodopapositron emission tomography (PET), [11C]dihydrotetrabenazinePET, [123I]beta-carbomethoxy-3beta-(4-iodophenyl) tropane single photon emission CT (DAT-SPECT), and [18F]fluorodeoxyglucose PET, have been proposed as markers for nigral abnormalities, disease progression or clinical characteristics for PD [36, 37]. For instance, longitudinal studies have shown an annual rate of reduction in striatal DAT uptake of 6–13% in PD patients [38, 39]. However, these examinations are invasive, expensive, and there is still uncertainty on whether there is an interaction between results and therapeutic intervention outcomes. For this reason, these neuroimaging techniques are not commonly used for routine diagnosis or follow-up of PD patients. Moreover, a very recent study has shown a correlation between striatal DAT density, as measured by DAT-SPECT, and SN-NM volume loss [33]. On the other hand, transcranial ultrasound has also been shown to detect increased echogenicity in the SN in PD as an indirect measure of neuronal loss [40], but this technique is limited by the requirements of a good temporal bone window and its ability in tracking disease progression is still unclear. Recently the loss of the "swallow tail" in the dorsolateral SN

as observed at high resolution 3T – SWI MRI has been proposed as an *in vivo* diagnostic biomarker for nigral degeneration in PD [14]. However even if such a radiological assessment yielded a high diagnostic accuracy (sensitivity 100%, specificity 95%), no longitudinal studies have investigated its modification with disease progression.

Our study has several limitations namely the small number of patients in each group and the cross-sectional nature with no longitudinal follow-up. On the other hand, our results clearly show a significant NM signal area reduction in PD patients compared to controls and a tendency for an NM area decrease along with disease progression. These findings are consistent with previous reports and validate the consistency of our results. Due to the small number of patients we were not able to investigate the age-related effect on NM area reduction throughout other statistical techniques (stratification nor regression model). However, no correlation was found between age and area, suggesting a more probable role of disease stage on NM area reduction. NM-MRI has also several technical characteristics that have to be considered when evaluating the feasibility of performing related imaging studies. It requires a long acquisition time, and the images suffer from relatively low spatial resolution, in-plane signal inhomogeneity and not all image analysis processes are completely automated, although few operator-dependent steps are required. Moreover, motion artifacts during image acquisition and partial volume effects may deteriorate the quantitative nature of the analyses. Nevertheless, we succeeded in performing MRI on all subjects without problems, obtaining good quality images and semi-automated analysis was possible for all patients. Finally there have been, so far, no reproducibility studies of NM-sensitive MRI. However, there have been up to now several studies using this specific sequence with different equipment and the obtained results are similar in terms of the identification of SN changes in PD patients [15, 25], which is strongly supporting sequence reliability.

Conclusions

In the present study, with semi-automated MRI measures, we detected a stage-dependent progressive decrease in the SN-NM area of PD patients. A marked SN-NM area decrease occurred in parallel with other markers of disease severity. Our findings suggest that NM-sensitive MRI could be used as a potential biomarker for nigral degeneration and disease progression in PD patients. Furthermore, to the best of our knowledge, this is the first study that observed SN-NM area modifications in a sample of LSPD patients, allowing an assessment of the modifications of NM signal in very late disease stage. CR values, although showing a tendency for a decrease with disease progression, presented a slight, albeit not significant, increase in the LSPD group; its interaction with therapeutic intervention and its modifications with disease progression needs further investigation.

Further longitudinal studies on a larger population and the use of consensus acquisition and analysis protocols are warranted in order to replicate our results, verifying if SN-NM area can measure PD patients' progression and if it could be considered as a disease progression imaging biomarker in clinical trials.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest relevant to this work.

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Editor's Comment: The study by Fabbri et al sheds some light on the familiar complaint by patients with PD that as they get older their medicine becomes less effective. The authors studied 20 patients whose mean age fell just shy of 80 (disease duration 14 years), and compared their response to a dopamine challenge with a group of PD patients who also had long disease duration (18 years), but were younger, and had undergone DBS. After the L-dopa challenge (mean dose 315 mg) the improvement on the MDS-UPDRS-III was only 11% (median UPDRS score of 57). By contrast, the DBS group improved by 37%. Of note, the late-stage patients were troubled by both somnolence and dyskinesias following the challenge. The authors propose that late-stage Parkinson's disease (LSPD) be considered to be a distinct clinical stage.

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Do patients with late-stage Parkinson's disease still respond to levodopa?

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ABSTRACT

Background: Late-stage Parkinson' disease (PD) is dominated by loss of autonomy due to motor and non-motor symptoms which can be marginally corrected by medications adjustments. However, controversy exists on the mechanisms underlying the apparent decrease of benefit from levodopa.
Objective: To study the response to levodopa in late-stage PD (LSPD).
Methods: 20 LSPD patients (Schwab and England ADL Scale <50 or Hoehn Yahr Stage >3 in MED ON) and 22 PD patients treated with subthalamic deep brain stimulation (DBS) underwent an acute levodopa challenge test. MDS-UPDRS-III and the modified Abnormal Involuntary Movement Scale were evaluated in off and after administration of a supra-maximal levodopa dose.
Results: LSPD patients had a median age of 78.8 (IQR: 73.5–82) and median disease duration of 14 years (IQR: 10-19.75). DBS patients had a median age of 66 (IQR: 61-72) and median disease duration of 18 years (IQR: 15-22). LSPD and DBS patients' MDS-UPDRS-III score improved 11.3% and 37% after levodopa, respectively. Rest tremor showed the largest improvement, while axial signs did not improve in LSPD. However, the magnitude of levodopa response significantly correlated with dyskinesias severity in LSPD patients. One third of LSPD and 9% of DBS patients reported moderate drowsiness.
Conclusions: LSPD patients show a slight response to a supra-maximal levodopa dose, which is greater if dyskinesia are present, but it is frequently associated with adverse effects. A decrease in levodopa response is a potential marker of disease progression in LSPD.

1. Introduction

Patients with Parkinson's disease (PD) develop levodopa-induced motor complications (MCs) after long-term levodopa (L-dopa) treatment [1]. The development of MCs usually defines the beginning of the advanced disease stage [2]. A number of advanced PD patients enter a later stage when motor and non-motor symptoms (NMS) symptoms such as falls and dementia start having a major impact on the health status of patient [3,4]. In comparison, MCs are less disabling in this late phase [3].

Recently, we have reported on the clinical characteristics and disabilities of a hospital-based population with late-stage PD

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(LSPD) [4–6], highlighting that some of these patients have to decrease dopaminergic therapy due to the occurrence of adverse effects (AEs). This raises the question whether the worse motor state of LSPD patients is due to the down-titration of L-dopa because of AEs or decline of levodopa responsiveness due to disease progression.

In order to investigate this, we report here the response of a LSPD population to an acute L-dopa challenge test.

2. Patients and methods

2.1. Objective

To study the motor response of a LSPD population to an acute L-dopa challenge test.

2.2. Study design and patients recruitment

This was a cross-sectional study in idiopathic PD patients according to the UKBB criteria [7]. Patients were included in the LSPD group if they had a Schwab and England score (S&E) [8] <50 or a Hoehn Yahr Stage (HY) > 3 in MED ON. The rating of the S&E scale was done by the clinician interviewing the patient and the caregiver. As an "active control group", we used an advanced stage PD group, defined as patients treated with sub-thalamic nucleus deep brain stimulation (STN-DBS) at least three years before and who did not fulfill the criteria of LSPD. Patients were consecutively recruited from the Movement Disorders outpatient clinic of a tertiary university hospital (Hospital Santa Maria, Lisbon, Portugal). The Local Ethical Committee approved the study and all patients provided informed consent.

2.3. Patients assessment

LSPD patients were first assessed at least 12 h after the last L-dopa/aromatic amino acid decarboxylase inhibitor (LDDCI) intake, 48 h after the last intake of dopamine agonists, controlled-release LDDCI, selegiline or rasagiline, or 12 h after the last intake of entacapone (practically defined "MED OFF"/"Condition A″); then, patients were assessed 60–90 min after or in the best "MED ON" ("Condition B") condition after a L-dopa intake. For the L-dopa challenge test, each patient took her/his usual morning L-dopa equivalent dose plus 50% (supra-maximal dose = 150%). L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversions [9]. Assessments were performed at patients' home whenever required by patients' health status or caregiver preference.

DBS patients were first assessed in the practically defined "MED OFF" condition and with the neurostimulator switched OFF for at least 60 min (MED OFF/STIM OFF, "Condition A"). Then, they took the same L-dopa dose as they did in the L-dopa challenge test performed for DBS selection years before (supra-maximal dose), and were assessed again in their best ON (MED ON/STIM OFF, "Condition B").

Motor performance was evaluated using the MDS-UPDRS part III scale [10], the Modified Abnormal Involuntary Movement Scale (mAIMS) and the HY stage during both "Condition A" and "Condition B". Parkinsonism was considered asymmetric when right–left differences in resting tremor, bradykinesia and rigidity were ≥5 points on the MDS-UPDRS items 3.3, 3.4, 3.6, 3.8 and 3.15–3.17. We defined and stratified levodopa-induced MCs according to the following scores: presence of motor fluctuations (MDS-UPDRS 4.3 ≥ 1); troublesome motor fluctuations (MDS-UPDRS 4.4 ≥ 2); presence of dyskinesias (MDS-UPDRS 4.1 ≥ 1) and troublesome dyskinesias (MDS-UPDRS 4.2 ≥ 2). Presence of psychosis was considered if MDS-UPDRS 1.2 score ≥1. Clinical phenotypes were defined both in concordant clinical history and the algorithm proposed by Stebbins and coworkers [11].

Both the patient and the investigator completed the Clinical Global Impression Severity Scale (CGI-S) before the L-dopa test and the Clinical Global Impression Improvement Scale (CGI-I) after the test.

Cognition and mood were assessed during "Condition B″, waiting until any L-dopa related limiting discomfort (e.g. nausea) improved, using the Portuguese version of the Mini Mental State Examination (MMSE) [12], the Geriatric Depression Scale (GDS), and the Pill Questionnaire. Diagnosis of PD with Dementia (PDD) was made according to the recommendation of the MDS Task Force [13]. Depression was diagnosed if a patient had a GDS score ≥11.

Data on demographics, clinical manifestations, disease management, co-morbidities and past medical conditions were obtained using a structured questionnaire (interviewing patients and caregivers), MDS-UPDRS part I, II and IV [10], and review of medical charts when needed.

3. Statistical Analysis

Descriptive statistics of demographic, clinical and therapeutic data were provided for continuous [median and interquartile range (IQR, 25th–75th percentile)] and categorical (count and percentage) variables.

The acute effect of L-dopa on motor symptoms was calculated comparing the MDS-UPDRS-III score and the mAIMS during "Condition A" versus "Condition B", using the Wilcoxon signed ranked test or the Fischer's exact test, as appropriate. The magnitude of response to levodopa was calculated as MDS-UPDRS-III during MED OFF minus MDS-UPDRS-III during MED ON/MDS-UPDRS-III during MED OFF. The Δ MDS-UPDRS-III was defined as the MDS-UPDRS-III during MED OFF minus MDS-UPDRS-III during MED ON.

MDS-UPDRS-III sub-items for speech (item 3.1), resting tremor (item 3.17), rigidity (item 3.3), bradykinesia (sum of items: 3.4–3.8 and 3.14), posture (item 3.13), gait (item 3.10), freezing of gait (item 3.11), arising from chair (item 3.9), postural instability (item 3.12) and total axial signs (sum of items: 3.1, 3.10–3.12) were studied separately.

Spearman's rank correlation coefficient was used to assess the correlation between the response to L-dopa (Δ MDS-UPDRS-III) with a history and severity of motor fluctuations and\or dyskinesias measured by the MDS-UPDRS IV total score, the MDS-UPDRS items 4.3 plus 4.4 for motor fluctuations and the items 4.1 plus 4.2 for dyskinesias, and with acute onset of L-dopa induced dyskinesias (LIDs) measured by the ΔmAIMS.

Descriptive statistics are reported for the response to L-dopa challenge test for both LSPD and DBS groups, however no direct statistical comparison was done between both groups as the study was not designed as a case-control study. Indeed LSPD and DBS patients were not matched for any relevant variables (e.g. age, disease duration, duration of levodopa treatment, etc.) refraining the possibility to perform a direct comparison. The advanced stage PD group was used as an active control group, included to better inform the analysis and interpretation of the results from the LSPD patients.

P value < 0.05 was considered significant. SPSS 21.0 statistical software (SPSS, Chicago, IL) was used.

4. Results

4.1. Demographic and clinical data

Forty-two patients were included in the study: 20 LSPD and 22 DBS patients (Demographic and clinical data in Table 1). Seventeen

Table 1
Demographic and clinical characteristics of LSPD and DBS patients. Values are presented as median [IQR, 25th–75th percentile] if no otherwise specified. GDS: Geriatric Depression Scale ((mild depression: 11–20; severe depression: 21–30). BMI: Body max index; MMSE: Mini Mental State Examination. Missing data: (*) → GDS 3/20; CGI-S (patients): 7/20. PIGD: postural instability/gait difficulty. Criteria I: clinical history; Criteria II: Stebbins et al., 2013. ND: not determined; NA: not available. ˆ: One LSPD patient was HY 2 due to very severe freezing of gait and speech that had a marked impact on ADL.

Patients data	LSPD (n = 20)		DBS (n = 22)	
Age (yrs)	78.8 [73.5–82]		66 [61–72]	
Education (yrs)	4 [3.25–7]		4 [4–7]	
Women (n/total (%))	11/20 (55%)		12/22 (54%)	
BMI (Kg/m^2)	20.4 [18.5–25.1]		26.1 [24.3–30.2]	
Age at disease onset (yrs)	65.5 [53.5–69.5]		48 [38–54]	
Disease duration (yrs)	14 [10–19.75]		18 [15–22]	
Levodopa treatment duration (yrs)	13 [9.75–20]		16 [12–21]	
Months after DBS	/		57 [44–68]	
Age at DBS (yrs)	/		62 [57–68]	
Asymmetric disease (n (%))	1 (5%)		2 (9%)	
S&E (ON/OFF)	40/30 [30–40/20–30]		90/85 [70–90/67–90]	
HY (ON/OFF)	4/4		2/2	
HY stage in ON (n (%))	2 = 1 (5%) 3 = 2 (10%) 4 = 15 (75%) 5 = 2 (10%)		1 = 2 (9%)ˆ 2 = 19 (87%) 3 = 1 (4%)	
Clinical phenotype (n (%))	**Criteria I**	**Criteria II (OFF/ON score)**	**Criteria I**	**Criteria II (OFF/ON score)**
Akinetic-Rigid	11 (55%)	NA/NA	12 (54%)	NA/NA
Tremor dominant	9 (45%)	9/0 (45%–0%)	7 (32%)	1/0 (4%–0%)
Mixed	0	NA/NA	3 (14%)	NA
PIGD	0	8/20 (40%–05)	0	20/20 (90%)
ND	NA	3/0 (15%–0%)	NA	1/2 (4%–9%)
PDD (n (%))	14 (70%)		0	
MMSE	20 [16.5–25.5]		29 [27–30]	
Psychosis (n (%))	9 (45%)		4 (18%)	
GDS	18 [15–19.5]*		13 [6.7–19.5]	
Depression (n (%))	14 (82%)		13(59%)	
Light	12 (70%)		6 (27%)	
Severe	3 (17%)		7 (32%)	
CGI-S (investigator)	6 [5–6]*		3 [2.7–4]	
CGI-S (patient)	5 [4–6]*		3 [3–3.2]	
MDS-UPDRS-I	23 [20–27.5]		14.5 [11.5–24]	
MDS-UPDRS-II	36 [31.2–40.7]		18.5 [13.7–23.5]	
MDS-UPDRS-IV	4 [0.2–7.7]		2.5 [0–8]	
MDS_UPDRS_III (OFF)	67 [60.5–78.2]		52.5 [42–57.5]	
MDS-UPDRS-III (ON)	57 [50.2–64]		19.5 [14–31.2]	
L-dopa induced Motor complications (n(%))	15 (75%)		15 (68%)	
Motor fluctuations (n (%))	11 (55%)		10 (45%)	
Troublesome motor fluctuations (n (%))	8 (40%)		7 (31%)	
Dyskinesias (n (%))	9 (45%)		13 (59%)	
Troublesome Dyskinesias (n (%))	5 (25%)		9 (40%)	

Table 2
Disability and disease severity milestones of LSPD patients.

	LSPD (N = 20) Num/total (%)
Gait and walking aid	
Independent	0 (0%)
Cane	3/20 (15%)
Walker	8/20 (40%)
Another person	7/20 (35%)
Weelchair-bound	2/20 (10%)
Falls (last month)	6/20 (30%)
Num/month (median [IQR])	3 [2–5]
Psychosis	9/20 (45%)
Neuroleptic treatment	5/20 (25%)
Neuroleptic treatment without psychosis	2/20 (10%)
PDD	14/20 (70%)
taking rivastigmine \ memantine	7/14 (50%)
Dwelling place	
Home	12/20 (60%)
Home & daytime residential	2/20 (10%)
Nursing home	6/20 (30%)
Time from admission (months) (median [IQR])	48 [IQR: 11-63]
Time to admission (yrs) (median[IQR])	11 [8–26]
CAREGIVER	
Informal (home)	7/20 (35%)
Formal (home)	7/20 (35%)
Formal (Residential care)	6/20 (30%)

LSPD patients (85%) were observed at home or nursing home due to severe disability. Disability milestones of LSPD patients are detailed in Table 2 while therapeutic data are depicted in Table 3.

4.2. Levodopa acute challenge test

4.2.1. LSPD patients

The median L-dopa dose for the test was 315 mg [IQR: 277-375]. The median MDS-UPDRS-III score was 67 [IQR: 60.5–78.2] in MED OFF and 57 [IQR: 50.2–64] in MED ON, with a significant median improvement of 11.3% [IQR: 6%–23%] ($p < 0.001$) (Table 4). Sub-analysis of MDS-UPDRS-III scores showed a significant median improvement after L-dopa intake for the following sub-items: "rest tremor" 0% [IQR: 0%–93%] ($p < 0.05$),"rigidity" 34% [IQR: 7%–87%] ($p < 0.001$),"bradykinesia" 11% [IQR: 0%–19%]($p < 0.001$). For the 9 patients with rest tremor, the median improvement was 100% [IQR: 12.5%–100%]. Overall Gait had a minimal, but still significant improvement ($p = 0.046$); this median benefit was 25% [IQR: 25%–31%] in those four patients showing improvement of gait after L-dopa. No significant improvement was found for all other axial signs (Table 4).

Half of the LSPD patients presented LIDs ($p < 0.005$ for mAIMS), which were generalized in 40% of the cases, involving the lower

Table 3
Therapeutic data of the patients. LEDD: Levodopa equivalent daily dose. (*): Stimulation frequency was 130 Hz and pulse width was 60 μs for all patients (except for one patient who had a pulse width of 90 μs). All patients were on monopolar stimulation except for one patients who had bipolar stimulation. The median reduction of LEDD was 57% (IQR: 26.5%–65%) after 57 months of DBS.

Medication	LSPD (N = 20)	DBS (N = 22)
Levodopa (n (%))		
Total	20 (100%)	16 (72%)
Monotherapy	17 (85%)	5 (22%)
Combination	3 (15%)	13 (65%)
LEED (Median [IQR])	912.5 [760–1160]	555 [312–720]
No anti parkinsonian medication	0	1 (4.5%)
Agonists (n (%))	0	12 (54%)
Total		2 (9%)
Monotherapy		
Amantadine (n (%))	1 (5%)	3 (13%)
Entacapone (n (%))	1 (5%)	1 (4.5%)
Selegiline/Rasagiline (n (%)	1 (5%)	5 (22%)
Neuroleptics (n (%))	5 (25%)	1 (4.5%)
Benzodiazepines (n (%))	8 (40%)	14 (63%)
Antidepressants (n (%))	7 (35%)	13 (59%)
Rivastigmine (n (%))	5 (25%)	0
Quetiapine (n (%))	4 (20%)	1 (4%)
Clozapine (n (%))	1 (5%)	0
Memantine (n (%))	2 (10%)	0
Non-neurological medication (n (%))	15 (75%)	11 (50%)
Stimulation Voltage (median [IQR])		
R_STN/L_STN*	/	3/3 [2.8–3.3]
LEDD before surgery		1015 [731–1635]
LEDD after surgery		555 [312–720]

Thirteen patients (65%) succeed in completing the CGI-I scale (median score: 4 – "no change"), while investigators' median score of the CGI-I was 3 ("minimally improved").

No serious AEs occurred during the test: 6 patients (30%) reported moderate drowsiness or fell asleep after levodopa, 5 of them reported sleep problems during the interview (MDS-UPDRS $1.7 \geq 1$).

4.2.2. Advanced stage PD patients

The median L-dopa dose for the test was 350 mg [IQR: 287-450]. The MDS-UPDRS-III total score improved significantly (37% [IQR: 26%–57%]) after L-dopa ($p < 0.001$), as did all sub-items with the exception of postural stability (Table 4). Sub-analysis of MDS-UPDRS-III scores showed a statistical significant median improvement of "speech" 0% [IQR: 0%–33%], "rest tremor" 50% [IQR: 0%–100%],"rigidity" 67% [QR: 0%–100%] ($p < 0.001$),"bradykinesia" 35% [IQR: 23%–55%], "gait" 25% [IQR: 0%–50%], "freezing" 25% [IQR: 0%–66%], "posture"0% [IQR: 0%–50%], "arising from chair" 0% [IQR: 0%–27%] (Table 4).

No statistically significant difference was found for the mAIMS. Neither the occurrence of LIDs (mAIMS during MED ON\STIM OFF) nor an history of drug-related MCs (MDS-UPDRS- IV) correlated significantly with the response to L-dopa. The median CGI-I score was 2 ("much improved") for both investigator and patients.

4.2.3. Late-stage PD versus advanced stage PD: response to levodopa

Even though no direct statistical comparison has been performed, the magnitude of response to L-dopa in LSPD patients was smaller than in the advanced cohort (Table 4), and this difference was even more marked on axial signs. In spite of a smaller motor response, the occurrence of L-dopa-related AEs was more frequent among LSPD patients.

Table 4
Values are presented as median [IQR, 25th–75th percentile]. mAIMS: modified Abnormal involuntary movement scale. Statistical significant results are in bold. Axial Signs: sum of item 3.1, 3.10–3.12 of the MDS-UPDRS-III. (*): S&E scores during ON and OFF condition were not evaluated before and after the levodopa challenge test but by means of the clinical interview. p*: MED OFF versus MEN ON; p°: MED OFF/STIM OFF versus MED ON/STIM OFF.

	LSPD patients (N = 20)				DBS patients (N = 22)			
	MED OFF	MED ON	Effect size (Δ)	p*-value	MED OFF/STIM OFF	MED ON/STIM OFF	Effect size (Δ)	p°-value
MDS-UPDRS-III	67[60–78.2]	57 [50–64]	8.5 [4.7–16.7]	**<0.001**	52.5 [42.5–58.2]	27 [20–37.5]	18.5 [14–27.5]	**<0.001**
Speech	3 [2–4]	3 [2–4]	/	**1**	3 [2–3]	2.5 [2–3]	0 [0–1]	**<0.05**
Rigidity	9 [4–14.25]	3.5 [0–11]	3.5 [1–4.25]	**<0.001**	4 [1–8.2]	0.5 [0–3]	3 [3–4]	**<0.001**
Bradykinesia	36,50 [33–40]	33 [24.2–37.5]	4 [0–6.5]	**0.001**	30 [24.7–32]	19 [11.7–23]	11 [7–16]	**<0.001**
Rest tremor	0 [0–4]	0	0 [0–2.2]	**<0.05**	2 [0–3]	0 [0–1]	1 [0–2]	**0.001**
Arising from chair	4 [3–4]	3.5 [3–4]	/	0.157	0 [0–2]	0 [0–1]	0[0–1]	**<0.05**
Freezing of gait	3 [2–3]	2 [2–2]	0 [0–0.5]	0.068	1 [0–3]	1 [0–1.2]	1 [0–1]	**0.05**
Posture	2 [2–3]	2 [2–3]	/	**1**	1.5 [1–2]	1 [1–2]	0 [0–1]	**<0.05**
Postural Stability	3 [3–4]	3 [3–3.75]	/	0.059	0 [0–1]	0 [0–0]	/	0.059
Gait	3 [3–4]	3 [3–3.75]	0 [0–0.5]	**<0.05**	2 [2–3]	2 [1–2]	1 [0–1]	**<0.001**
Axial Signs	19 [17–22.5]	17 [15–19]	0 [0–2]	0.053	6.5 [5–9]	5 [3–6.2]	2 [1–3]	**<0.001**
AIMS	0 [0–0]	1.5 [0–9.5]	1.5 [0–8.7]	**0.001**	0 [0–4]	1.5 [0–6]	0.5 [0–4.5]	0.13
S&E*	30 [20–40]	40 [30–40]	0 [0–10]	**<0.05**	85 [67–90]	90 [70–90]	**0** [0–10]	0.1
HY	4 [4–5]	4	0 [0–1]	**<0.05**	2	2	/	**1**
Occurrence of AEs	6 patients (30%) = drowsiness; 1 patients (5%) = symptomatic orthostatic hypotension				2 patients (9%) = drowsiness; 1 patients (4%) = hypertensive crisis			

limbs, neck or trunk in 35%, the face in 30% and the upper limbs in 25% of the cases. The dyskinesias were choreic and mild in 80% of the patients. We found a significant correlation between the ΔmAIMS and the Δ MDS-UPDRS-III score ($R = 0.581$; $p < 0.0.5$). Similarly, the MDS-UPDRS-IV total score and the presence of dyskinesias (items 4.1 plus 4.2) showed a significant correlation (respectively: $R = 0.67$; $p < 0.05$; and $R = 0.634$; $p = 0.05$) with the Δ MDS-UPDRS-III score, while no correlation was found with motor fluctuations alone (items 4.3 plus 4.4) ($p = 0.8$). A correlation was also found between the MDS-UPDRS-IV and the mAIMS ($R = 0.669$; $p < 0.05$). Notably, all patients with improved gait after L-dopa (4 patients) had a worse MDS-UPDRS-IV total score and MDS-UPDRS-IV item 4.3 score compared with those who did not have gait improvement ($p = 0.05$).

5. Discussion

As previously reported [3,5,14,15], our new sample of LSPD patients was severely disabled. Now we have found that these patients show a moderate response to a supra-maximal L-dopa dose, although this was frequently associated with the occurrence of AEs.

The response of LSPD patients to L-dopa is poorly understood and it has never been systematically analyzed. In a previous study [4], we have identified that a proportion of these patients have difficulties in increasing the dose of dopaminergic therapy, or even had to decrease it, due to AEs. We have now explored whether the motor severity occurring in LSPD is due to the down-titration of dopaminergic drugs, because of AEs, or levodopa-unresponsiveness due to disease progression. Additionally, we applied the same study protocol to a group of advanced stage PD patients that was used as an "active control group". It is acknowledged that DBS patients were selected for surgery because they have a long disease duration, good response to L-dopa and troublesome motor complications, thus they represent a selected group of advanced PD patients. The lack of data on acute L-dopa effect in LSPD patients suggested the evaluation of this group of patients with the same protocol allowing to better inform the interpretation of their results. An earlier PD population not meeting criteria for LSPD, could be also an informative alternative. Moreover we assumed by definition that advanced PD patients were substantially different from LSPD ones, being characterized by a higher L-dopa responsiveness and a lower frequency of dementia and psychosis. However the choice of an "active control group" was exclusively to inform and validate the results of the study, even though we were aware of the existence of "*a priori*" clinical differences between the two PD groups.

The motor response of LSPD patients was modest, represented an increase of 11.3% in MDS-UPDRS-III score. In contrast, a similar L-dopa dose induced a greater improvement (37%) in advanced PD compared to LSPD patients in spite of a higher BMI of the former which is generally associated to a reduced L-dopa's AUC [16], further, suggesting that there is a weaker response to an acute L-dopa dose in later stages of PD. However, based on patients' medical charts and clinical history, these LSPD patients had responded well to L-dopa in the past. Rest tremor was the limb symptom that responded best, followed by rigidity and then bradykinesia. Interestingly, this pattern of appendicular symptom response to L-dopa seems to follow that of earlier PD stages [17]. Although gait significantly improved, the median score was 3 in both MED OFF and MED ON, suggesting that this improvement was of no functional relevance. Similarly, other axial signs did not improve either, thus highlighting the resistance of axial signs to L-dopa therapy compared to earlier PD stages [17]. Axial symptoms classically worsen with disease progression [14,15,18] constituting one of the major sources of disability and they mostly become L-dopa unresponsive due to extranigral pathology [19].

Despite a statistically significant change of MDS-UPDRS-III score, L-dopa had no meaningful clinical implication in the LSPD patients at the CGI-I. Moreover the change in S&E from 30% in MED OFF to 40% in MED ON, although statistically significant, had very little impact on independence for patients. The lack of benefit perceived by patients is probably due to several factors.

First, the acute motor improvement may in fact be minimal and thus not meaningful for patients. Indeed, there is a minimal difference in the motor scores that is judged as clinically meaningful. This minimum clinically important change has been calculated for early PD patients in HY stage 1–3 after 6 months of treatment using the UPDRS and the CGI-I completed by the clinician [20]. Schrag and colleagues determined the minimum change to be a reduction of 5 points in the UPDRS motor score, but no data is available for more advanced stages [20]. Nevertheless, we speculate it would be higher than 5 points for LSPD, and although we found a median reduction of 8.5 points at the MDS-UPDRS-III, it may not be enough to be perceived as meaningful by LSPD patients, as they still had a high MDS-UPDRS motor score in ON.

The second factor potentially affecting the lack of benefit perceived by LSPD patients is their low ability to self-perceive and communicate their opinions due to cognitive decline, speech impairment and the occurrence of drowsiness after L-dopa intake. Finally, patients may conclude that the benefit they get with L-dopa is not strong enough to compensate for the occurrence of troublesome AEs.

We found a positive correlation between L-dopa response and the severity of dyskinesias or the acute onset of LIDs, as previously reported [21,22]. This suggests that only patients with dyskinesia might gain an additional benefit from L-dopa increment. This probably occurs because dopamine receptors are still sensitive to L-dopa stimulation in these individuals [23]. However little is known about the pre and post-synaptic functional status of LSPD patients who do not respond to L-dopa at all, particularly whether it is related to striatal cell death. It is likely that the change in motor response to L-dopa in late PD stages is not solely due to presynaptic nigrostriatal dopaminergic dysfunction, but also to extra-nigral alterations. Indeed, a loss of striatal dopamine D3 receptors has been correlated with loss of response to dopaminergic drugs and presence of dementia in PD [24] and striatal dopaminergic neurons seem to undergo structural changes and death with disease progression [25,26]. Moreover, extra-striatal pathology such as the involvement of the pedunculopontine nucleus in Braak stage 3 [27] may underlay postural instability and gait disorder. The absence of severe dyskinesias in LSPD patients during the L-dopa test may be an additional sign of a blunted response to L-dopa.

Notably a third of LSPD patients showed a moderate somnolence during the test while only two DBS patients reported drowsiness in spite of a slightly higher L-dopa dose, suggesting that some L-dopa-related AEs may increase with disease course.

Finally, we have found that LSPD patients have great difficulty in completing several scales, highlighting the hurdles that investigators can face and the lack of proper disease rating scales adjusted to this population disability.

DBS patients had a statistical significant improvement after the acute L-dopa test in all motor sub-items, with the exception of postural stability. This is in accordance with the results of several studies finding a progressive decrement of L-dopa effect in DBS patients with medium/long-term post-surgical follow-up, especially for axial signs [28,29]. An additional bias that could have enlarged the difference in L-dopa responsiveness between LSPD and advanced PD patients is the younger age at onset for DBS patients. Indeed it has been shown an increased risk of LIDs in patients with disease onset before the age of 55 and we know that PD patients with earlier motor fluctuations usually present a stronger response to L-dopa and better motor improvement [22,21]. An interesting finding in our DBS group is the lack of a statistically significant development of dyskinesias after L-dopa intake, supporting the idea that chronic STN high frequency stimulation may induce pharmacodynamic changes and increase the threshold for dyskinesias promoting desensitization to LIDs [30,31].

5.1. Study limitations

Additional limitations to those addressed above are the small sample size, the unblinded clinical assessments for both patients group's allocation and medication/stimulation conditions, lack of previous data on acute L-dopa effect in LSPD patients and a short wash-out period for the STIM OFF condition.

We were aware of those limitations during protocol design and accordingly we consider ours an exploratory study that needs future validation. However, to our knowledge, this is the first study that explores the response to an acute L-dopa challenge test in late phase PD. We cannot exclude a stimulation carry-over effect due to the short wash-out period of stimulation. Nevertheless a longer one would probably not be tolerable to patients.

6. Conclusion

In spite of its huge impact on health care systems, LSPD remains an orphan population, barely reached by movement disorder specialists and poorly investigated, but whose prevalence is expected to increase in the near future. This exploratory study shows that LSPD patients still show a slight response to a supra-maximal L-dopa dose, though this is frequently associated with troublesome AEs. Resting tremor, followed by bradykinesia and rigidity are the main motor features that improve with L-dopa, while axial signs do not change, with the exception of gait in few patients. Even in this late stage, patients manifesting MCs are the ones most responsive to L-dopa [20,21]. We suggest an increase in the dose of L-dopa in those LSPD patients manifesting MCs in whom tremor or rigidity are the most troublesome motor symptoms. We acknowledge however that an acute benefit with L-dopa may not translate into a long-term improvement and drowsiness may not occur if L-dopa dose is increased slowly. Equally we are aware on the difference between acute and chronic L-dopa response, warning that stopping completely the L-dopa therapy could slowly and severely aggravate some motor symptoms among LSPD patients.

Our results also suggest that loss of acute responsiveness to L-dopa even in appendicular symptoms might be a sign of disease progression [20]. Finally, the development of better assessment tools that adjust to LSPD patients is a challenge for future clinical research.

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Authors' contributions

1. Research project: A. Conception, B. Organization, C. Execution; 2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique.

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Response of non-motor symptoms to levodopa in late-stage Parkinson's disease: Results of a levodopa challenge test

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ABSTRACT

Background: Non-motor symptoms (NMS) are extremely common among late-stage Parkinson's disease (LSPD) patients. Levodopa (L-dopa) responsiveness seems to decrease with disease progression but its effect on NMS in LSPD still needs to be investigated.
Objective: To assess the response of blood pressure (BP), pain, fatigue and anxiety to L-dopa in LSPD patients.
Methods: 20 LSPD patients, defined as Schwab and England ADL Scale <50 or Hoehn Yahr Stage >3 (MED ON) and 22 PD patients treated with subthalamic deep brain stimulation (advanced PD group) underwent an L-dopa challenge. BP and orthostatic hypotension (OH) assessment, a visual analogue scale (VAS) for pain and fatigue and the Strait Trait Anxiety (STAI) were evaluated before and after the L-dopa challenge.
Results: Systolic BP dropped significantly after L-dopa intake ($p < 0.05$) in LSPD patients, while there was no change in pain, fatigue or anxiety. L-dopa significantly improved ($p < 0.05$) pain and anxiety in the advanced PD group, whereas it had no effect on BP or fatigue. L-dopa-related adverse effects (AEs), namely OH and sleepiness, were more common among LSPD patients. 40% and 65% of LSPD patients were not able to fill out the VAS and the STAI, respectively, while measurement of orthostatic BP was not possible in four LSPD patients.
Conclusions: This exploratory study concludes that some non-motor variables in LSPD do not benefit from the acute action of L-dopa while it can still induce disabling AEs. There is a need for assessment tools of NMS adapted to these disabled LSPD patients.

1. Introduction

Parkinson's disease (PD) is a multisystem disorder characterized by several motor and non-motor symptoms (NMS) [1]. NMS are very common in PD, and their frequency and, in the majority of cases, their severity increase in more advanced stages [2,3]. Interestingly, the presence, and above all, the severity of levodopa (L-dopa)-induced motor complications (MCs) seem to decrease in late-stage PD (LSPD) [2,4], thus probably accounting for the major impact that NMS have on patients' quality of life (QoL). Although frequently underdiagnosed [5], NMS play a major role in the QoL of PD patients and carers [6]. Moreover, 30% of PD patients consider L-dopa-induced non-motor fluctuations more disabling than motor fluctuations [7].

The management of NMS is challenging throughout the disease course [8], but even more so during the later stages during which patients usually have to decrease dopaminergic therapy due to the

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occurrence of adverse effects (AEs) [9]. Overall, L-dopa responsiveness seems to decrease with disease progression, but very few studies have investigated L-dopa responsiveness among LSPD patients [4,10], and even less the benefit of L-dopa on NMS. To assess this, we report the response of NMS to an acute L-dopa challenge in a population of LSPD. To better understand the relevance of the results, a group of advanced stage PD patients submitted to subthalamic nucleus deep brain stimulation (STN-DBS) underwent the same protocol.

2. Patients and methods

2.1. Objectives

Our primary objective was to assess the response of blood pressure (BP), pain, fatigue and anxiety following an acute L-dopa challenge in an LSPD population.

2.2. Design and recruitment

We performed a cross-sectional study in a consecutive sample of LSPD patients, recruited during 6 months from the movement disorders outpatient clinic of a tertiary university hospital (Hospital Santa Maria, Lisbon, Portugal). PD was defined according to the UK Brain Bank criteria [11], whereas LSPD was defined as PD patients with either a Schwab and England score (S&E) < 50 (MED ON) or a Hoehn & Yahr stage (HY) > 3 (MED ON). A group of advanced PD patients was included as an "active control group", to better enlighten the interpretation of both the applicability of the assessment tools and the results. Advanced PD patients were defined as patients treated with STN-DBS at least three years previously, and who did not fulfill the criteria for LSPD. Patients who had undergone DBS were excluded from the LSPD group. The Local Ethical Committee approved the study and all patients provided informed consent.

2.3. Assessment of patients

LSPD patients were first assessed in the practically defined "MED OFF" condition and then 60–90 min after L-dopa intake in the best "MED ON" condition [11]. Each patient took her/his usual morning L-dopa equivalent dose plus 50% (supra-maximal dose = 150%). L-dopa equivalent daily dose (LEDD) was calculated according to recognized standard conversions [12].

Advanced patients were first assessed in the practically defined "MED OFF" condition and with the neurostimulator switched OFF for at least 60 min (MED OFF/STIM OFF), and then after taking the same L-dopa dose as they did in the L-dopa challenge performed for DBS selection years before (MED ON/STIM OFF). The protocol of the L-dopa challenge performed for DBS selection was the same as for LSPD patients, as previously reported [10].

NMS were evaluated using the MDS-UPDRS part I [13], the Non-Motor Symptoms Assessment Scale for PD (NMSS) [14], the Neuropsychiatric Inventory test 12-items [15], and the Geriatric Depression Scale (GDS) [16]. PD with Dementia (PDD) was diagnosed according to the recommendation of the MDS Task Force [17].

Depression was diagnosed if patients scored ≥11 on the GDS (mild depression between 11 and 20 points; severe depression between 21 and 30 points). Psychosis was present if patients had an MDS-UPDRS item 1.2 score ≥1.

Acute response of BP, pain, fatigue and anxiety to L-dopa were assessed immediately before and 60–90 min after L-dopa intake in the best "MED ON" condition. BP was measured in supine and 3 min after standing; orthostatic hypotension (OH) was defined as a decrease with standing in systolic blood pressure (SBP) > 30 mmHg or in diastolic BP (DBP) > 15 mmHg (criteria I), or in SBP >20 mmHg or in DBP >10 mmHg (criteria 2). Pain and fatigue were measured using a visual analogue scale (VAS; VAS-p for pain and VAS-f for fatigue). Anxiety was assessed with the State Trait of Anxiety Inventory (STAI), which is a psychological inventory consisting of 40 self-report items, 20 items to assess trait anxiety and 20 for state anxiety, each item is scored on a 4-point Likert-type response scale [18]. For the purpose of our study only the 20 items for state anxiety have been assessed. MDS-UPDRS motor part III [13]was performed in "MED OFF" and then best "MED ON" condition [10]. MDS-UPDRS parts II and IV were used to assess the impact of motor symptoms on activities of daily life and L-dopa-induced MCs, respectively [13].

3. Statistical analysis

Descriptive statistics of demographic, clinical and therapeutic data were provided for continuous [median and interquartile range (IQR, 25th–75th percentile)] and categorical (count and percentage) variables.

The acute effect of L-dopa on NMS was calculated by comparing the median value of BP and the development of OH, and the scores of VAS-p, VAS-f and STAI between MED OFF versus MED ON conditions for LSPD patients and between MED OFF/STIM OFF with MED ON/STIM OFF conditions for DBS patients. Comparisons were made using the Wilcoxon's signed-ranked test or the Fischer's exact test, as appropriate.

Spearman's rank correlation coefficient was used to assess the association between the magnitude of motor (Δ MDS-UPDRS-III) and NMS (ΔVAS-p and Δ VAS-f and Δ STAI) response to L-dopa, and the association between the severity of motor symptoms (MDS-UPDRS-III) and NMS (MDS-UPDRS-I, NMSS NPI-12 items and GDS). Two group comparisons were performed using Fisher's exact test (categorical variables) and the Mann-Whitney *U* test (continuous variables), as appropriate.

LSPD and DBS patients were not matched for relevant variables (e.g., age, disease duration, duration of L-dopa treatment, etc.) thereby not allowing for the possibility of performing direct comparisons between groups, although descriptive statistics are reported. A P value < 0.05 was considered significant. The software SPSS 21.0 (SPSS, Chicago, IL) was used.

4. Results

4.1. LSPD patients

4.1.1. Clinical data and NMS characteristics

20 LSPD patients were included in the study. All had had good response to L-dopa in the past. Demographic, clinical, disability milestones, and therapeutic data of these patients have been reported previously [10] and are summarized in Table 1. The application of patients' self-reported scales was hampered due to the presence of dementia and weak cooperation (Tables 1 and 2).

NMS were very frequent and affected all domains (Table 1). PDD was diagnosed in 70% of the patients and hallucinations and psychosis were present in 45% of the cases. Depression was very frequent according to the GDS (88%) and 35% of all cases were taking antidepressants (Table 1).

The overall severity of NMS was moderate-high (MDS-UPDRS part I items scoring ≥ 2 points), namely "cognition", "depressed mood", "anxious mood", "apathy", "day-time sleepiness", "urinary problems", "pain", "light-headedness and fatigue". The NPI-12 documented the presence of "agitation/aggression", "irritability/lability" and "aberrant motor behavior" in about one-third of the patients. In the NMSS the domains of "mood", "memory", "urinary",

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"sleep/fatigue", "gastrointestinal" and "sexual" were universally affected (Table 1). The frequency of several NMS was similar across the MDS-UPDRS part I, the NPI-12 and the NMS scales (Table 1).

The caregivers of six patients (30%) reported that their relative frequently spent several hours per day in a sort of apathetic state, with their eyes closed but apparently not asleep, as they replied if questioned. Among these patients, five (25%) reported the frequent occurrence of a "drowsiness state" 30–40 min after L-dopa intake, while anxiety occurring 15–30 min before L-dopa intake was reported by two patients.

4.1.2. Levodopa acute challenge test

The median L-dopa dose for the test was 315 mg [IQR: 277–375]. The median MDS-UPDRS part III score was 67 [IQR: 60.5–78.2] in MED OFF and 57 [IQR: 50.2–64] in MED ON, with a significant improvement of 11.3% [IQR: 6%–23%] ($p < 0.001$) (Table 2).

Measurement of BP in orthostatism was not possible in four patients (20%) due to their difficulty in remaining in a standing position. Median change of SBP was statistically different between MED OFF versus MED ON ($p < 0.005$). Three and four patients (according to criteria I and II, respectively) developed OH in MED ON, which was symptomatic in only one (Table 2).

Twelve patients (60%) succeeded in completing the VAS scales and 7 (35%) completed the STAI. Pain, fatigue and anxiety did not change significantly after L-dopa intake. There was no correlation between either the ΔVAS-p or ΔVAS-f and the ΔMDS-UPDRS part III while the ΔSTAI correlated with the ΔMDS-UPDRS part III ($R = 0{,}686$; $p < 0.005$). The score of the STAI was not significantly different between fluctuators (score of MDS-UPSRS part IV item $4.3 \geq 1$) and non-fluctuators. Moderate correlation was found between MDS-UPDRS part III (MED ON) and MDS-UPDRS part I ($R = 0{,}675$; $p < 0.05$), GDS ($R = 0{,}634$; $p < 0.005$) and NMSS ($R = 0{,}695$; $p < 0.05$), but not with NPI-12 items, indicating that a worse motor condition was associated with more severe NMS. Severity of motor parkinsonism was not significantly different between demented and non-demented patients, whereas PDD patients had worse scores of MDS-UPDRS parts I and II compared to non-demented patients.

No serious AEs occurred during the test. Six patients (30%) reported moderate drowsiness or fell asleep after L-dopa. The occurrence of L-dopa-related AEs was neither associated with longer disease duration, older age, age at PD onset, PDD, L-dopa dose, nor with a worse motor score (MED ON).

4.2. Advanced PD patients

4.2.1. Clinical data and NMS characteristics

22 DBS patients were included in the study and, overall, NMS were less severe in advanced patients compared to LSPD (Table 1). No advanced patient was demented, 18% reported hallucinations and depression was diagnosed in 59% of patients. The following items scored ≥2 points in the MDS-UPDRS part I, indicating moderate-high severity: "depressed mood", "anxious mood", "apathy", "pain", "urinary problems", "constipation" and "fatigue". Interestingly, joint and skeletal deformities were absent.

4.2.2. Levodopa acute challenge test

The median L-dopa dose for the test was 350 mg [IQR: 287–450]. The MDS-UPDRS-III score improved significantly (52.5 versus 27; 37% [IQR: 26%–57% $p < 0.001$]) after L-dopa (Table 2).

The intake of L-dopa had no significant effect on mean BP and fatigue. Four and five patients (according to criteria I and II, respectively) developed asymptomatic OH in MED ON (Table 2). L-dopa improved pain and anxiety ($p < 0.05$). The ΔVAS-p did not correlate with ΔMDS-UPDRS-III. On the other hand, the ΔSTAI had a moderate correlation with the magnitude of L-dopa response ($R = 0{,}427$; $p < 0.05$) but not with presence of "wearing-off" or "dyskinesias" (MDS-UPDRS-IV item 4.3 and 4.1). A moderate correlation was found between MDS-UPDRS part III (MED ON/STIM OFF) and the NMSS ($R = 0{,}427$; $p < 0.05$) but no correlation was found with the MDS-UPDRS part I or the NPI-12.

5. Discussion

As previously reported, we found a high frequency and severity of NMS among LSPD patients [3,6,19], which were correlated with motor disability. All domains of NMS were involved and most domains affected all patients. Frequency of NMS was similar among different scales, giving internal consistency to our results. We were able to perform an L-dopa challenge on these very disabled patients, although the difficulty encountered by patients completing the self-reported scales possibly hampered the assessment of the response of NMS. Despite this, the results showed no significant effect of an acute L-dopa challenge on pain, fatigue or anxiety, while SBP decreased after L-dopa intake and OH emerged in about 25% of tested patients. Additionally, AEs occurred in one-third of patients after the intake of L-dopa, namely sleepiness. Furthermore, we applied the same study protocol to a representative group of advanced stage PD patients who were used as an "active control group". The lack of data on acute L-dopa effect on NMS in LSPD patients suggested the need to assess this group of advanced PD patients in order to validate the assessment tools and enrich the results.

We decided to restrict the assessment of NMS only to some symptoms, namely pain, fatigue, anxiety and BP, the specific acute modifications of which could be evaluated during an L-dopa challenge in an in a frail population of LSPD population with a high frequency of dementia and speech difficulties and using relatively simple tools. Indeed, the majority of instruments available to assess NMS in PD may be inadequate in very disabled patients, similarly to other neurodegenerative conditions [20]. Such burden is a specific trait of LSPD patients, as we found no similar difficulties for the group of advanced PD patients. There is the additional risk of low reliability of LSPD patients' response to self-reported scales or questionnaires due to cognitive and speech impairments and the occurrence of AEs after L-dopa.

Nevertheless, we diagnosed probable dementia in 70% of LSPD patients, which is quite high compared to other case series (45%–50%) with similar disease duration [3,4], while the frequency of psychosis was similar to previous reports (about 45%) [3,4]. Depression was diagnosed in 88% of patients and the difficulty encountered in completing the GDS may have nevertheless resulted in an underestimation of its frequency and severity. The frequency of mild depression (70%) was found to be rather high, but almost half of the depressed patients were not taking antidepressants, which highlights how depressive symptoms may go unnoticed in such a late phase, or, alternatively, that antidepressants were discontinued in the past due to AEs. Dysautonomic symptoms were equally very frequent and bothersome to LSPD patients. The high frequency of daytime sleepiness, apathy and motor aberrant behavior in LSPD patients results in a severe clinical picture, in which patients spend most part of the day alternating between an "apathetic state" with eyes closed and periods of excessive sleepiness or purposeless motor behavior.

The acute L-dopa challenge induced a 23 mmHg drop in SBP and the occurrence of OH in one-fourth of patients. OH was symptomatic only in one patient, which contrasts with the high frequency of symptoms of orthostatism. Diagnosing and treating low BP in LSPD may prove beneficial in improving patients' handicap.

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Table 1
Demographic and clinical characteristics of LSPD and DBS patients. Values are presented as median [IQR, 25th–75th percentile] if no otherwise specified. LEDD: L-dopa equivalent daily dose; GDS: Geriatric Depression Scale. MMSE: Mini Mental State Examination. NMSS: Non-motor symptoms scale; NPI: Neuropsychiatric Inventory Scale; Missing data: (*) → GDS 3/20; the NPI was applied only to 16 LSPD patients and 18 DBS patients.

Demographics and clinical features	LSPD (n = 20)	DBS (n = 22)
Age (yrs)	78.8 [73.5–82]	66 [61–72]
Age at disease onset (yrs)	65.5 [53.5–69.5]	48 [38–54]
Disease duration	14 [10–19.75]	18 [15–22]
S&E (ON/OFF)	40/30 [30–40/20-30]	90/85 [70–90/67-90]
LEDD [IQR, 25th–75th percentile]	912.5 [760–1160]	555 [312–720]
HY (ON/OFF)	4/4	2/2
PDD (n (%))	14 (70%)	0
MMSE	20 [16.5–25.5]	29 [27–30]
MMSE (demented/non-demented)	18 [15–20.5]/26 [24.7–29.2]	//
Psychosis (n (%))	9 (45%)	4 (18%)
Neuroleptics treatment (n (%))	5 (25%)	1 (4.5%)
GDS Score [IQR, 25th–75th percentile]	18 [15–19.5]*	13 [6.7–19.5]
Depression (n (%))	15 (88%)	13 (59%)
Mild	12 (70%)	6 (27%)
Severe	3 (17%)	7 (32%)
Antidepressants treatment (n (%))	7 (35%)	13 (59%)
MDS-UPDRS-I	23 [20–27.5]	14.5 [11.5–24]
Score [IQR, 25th–75th percentile] – n° of patients scoring positive in the item (%)		
Cognition	4 [2–4] – 85%	1 [0–2] – 63%
Hallucinations &psychosis	0 [0–3]– 45%	0 [0–1] – 40%
Depressed mood	2 [1.2–3]– 80%	2 [1–3] – 81%
Anxious mood	2 [0–3]– 80%	2 [0–3] – 68%
Apathy	2 [1–3.7]– 70%	2 [1–2.2] – 86%
DDS	0–10%	0 [0–1] – 36%
Sleep problems	1 [0–2]– 65%	1 [0–2] – 63%
Daytime sleepiness	2 [2–2.7] – 90%	1.5 [1,2] – 77%
Pain	2.5 [0–3]– 70%	2 [0–3] –68%
Urinary problems	3 [2.2–3]– 100%	2 [1,2]– 81%
Constipation problems	1.7 [0–2–3.7]– 70%	2 [0–3]– 68%
Light headedness	2 [0.2–2] – 70%	1 [0–1.2]– 59%
Fatigue	3 [2–3.7] – 85%	2 [1–3]– 86%
MDS-UPDRS-II	36 [31.2–40.7]	18.5 [13.7–23.5]
MDS-UPDRS-IV	4 [0.2–7.7]	2.5 [0–8]
Painful off-dystonia, Score [IQR, 25th–75th percentile] – n° of patients scoring positive in the item (%)	0 [0–0.75] – 20%	0–18%
Joint and skeletal deformities (n (%))	4 (20%)	0%
NPI-12 items (total score)*	15 [3–23.5]	8 [2.5–16.5]
Score [IQR, 25th–75th percentile] – n° of patients scoring positive in the item (%)		
Delusion	0 [0–1] – 31%	0–0%
Hallucinations	0 [0–1.7] – 37%	0 [0–1] – 27%
Agitation/Aggression	0 [0–1] – 37%	0–5%
Depression	1.5 [1–4] – 87%	2.5 [0.7–4.5] – 77%
Anxiety	1 [0.2–4] – 75%	1 [1–4] – 66%
Elation/Euphoria	0–0%	0–0%
Apathy/indifference	4 [0.2–8.2] – 75%	1 [1–4.5] – 61%
Disinhibition	0–0%	0 –0%
Irritability/Lability	0 [0–1] – 31%	0–11%
Motor aberrant behaviour	0 [0–1] – 31%	0–0%
Sleep and Nighttime Behavior Disorders	2 [2–5.5] – 93%	1 [1–4] – 77%
Appetite and Eating Disorders	2 [2–5.5] – 25%	0 [0–1] – 44%
NMSS (total score)	120.5 [97.7–162.5]	63 [39.5–77]
Score [IQR, 25th–75th percentile] – n° of patients scoring positive in the item (%)		
Cardiovascular	4 [0–7] – 65%	1 [0–4] – 63%
Sleep/Fatigue	17 [8.2–21.5] – 100%	7 [2–12] – 91%
Mood/Cognition	23.5 [8.2–34.2] – 95%	11 [3–19.5] – 95%
Hallucination/perception	1 [0–12] – 50%	0 [0–2] – 32%
Memory	27 [6.7–36] – 100%	4 [0.7–7.2] – 77%
Gastrointestinal tract	7 [2.5–19.2] – 95%	5 [3–12] – 95%
Urinary	13 [9.2–24.7] –100%	3 [1–7.5] – 81%
Sexual function	24 [24–24] – 100%	14.5 [1–7.5] – 95%
Miscellaneous	11 [5.7–15.5] – 100%	8.5 [7.5–21.5] – 100%

Interestingly, L-dopa did not cause a significant decrease in BP in advanced PD patients, who had longer disease duration, suggesting that the severity of dysautonomia may not be determined solely by disease duration.

The intake of L-dopa did not significantly change the severity or the frequency of pain, fatigue and anxiety. This contrasts with the significant improvement of both anxiety and pain among advanced PD patients, possibly linked in part to their better motor response to L-dopa. Alternatively, the major source of pain in LSPD patients may be related to secondary causes such as radicular compression, musculoskeletal deformities and contractures, which do not respond to L-dopa and the treatment of which is challenging [9]. In fact, the frequency of painful off-dystonia, highly responsive to L-dopa, was similar for LSPD and DBS patients, but two-thirds of patients reported some discomfort due to pain, suggesting that other causes of pain could have a greater impact on patients [21,22].

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Table 2
NMS response to L-dopa. Values are presented as median [IQR, 25th–75th percentile]. STAI: State Trait of Anxiety Inventory (only the 20 items of state anxiety have been applied); VAS-p: visual analogue scale for pain; VAS-f: visual analogue scale for fatigue; BP_supine: blood pressure in supine position; BP_orto: blood pressure after 3 min of standing; OT: orthostatic hypotension HY: Hoehn Yahr; S&E: Schwab and England score; p°: MED OFF versus MEN ON; p*: MED OFF/STIM OFF versus MED ON/STIM OFF. **Missing values** – STAI: °3 over 20; VAS: * 8 over 20; BP: ° 4 over 20; 1-OH: defined as decrease in systolic pressure >30 mmHg and in diastolic pressure>15 mmHg, within 3 min of standing; 2-OH: defines as decrease in systolic pressure >20 mmHg and in diastolic pressure>10 mmHg, within 3 min of standing.

	LSPD patients (N = 20)				DBS patients (N = 22)			
	MED OFF	MED ON	Effect size (Δ)	p* - value	MED OFF/STIM OFF	MED ON/STIM OFF	Effect size (Δ)	p* -value
MDS-UPDRS-III	67.5 [60.6–78.2]	57 [49–64]	8.5 [4.5–16.7]	**<0.001**	52.5 [42.5–58.2]	27 [20–37.5]	18.5 [14–27.5]	**<0.001**
STAI	47.5 [41.2–52.7]°	41 [30–49]°	4 [0–22]°	0.1	50.5 [43.7–59.2]	37.5 [33–45]	13 [9–19.2]	**<0.001**
VAS-p	0 [0–4.5]*	0 [0–3]*	/	0.07	0 [0–5]	0	0 [0–3.5]	**<0.05**
VAS-f	5 [0–8]*	5 [0–5.7]*	/	0.2	2.5 [0–7]	1.5 [0–4.2]	0 [–2.5–5]	0.2
BP_supine	157/83 [135/83–174–90]	134/80 [111/78–170/95]	23 [1–38]/2.5 [-11–9]	**0.004**/0.7	147/90 [136/79–170/98]	145/90 [130/79–172/98]	/	1/0.133
BP_ortho	147/85 [127/69–178/93]°	105/75 [90/63–140/90]°	26 [0–49]/7 [–11–10]	**0.002**/0.2	147/93 [125/85–177/100]	139/89 [119/76–153/98]	12/5 [–9/24–5/20]	0.1
1-OH (n (%))	4 (20%)°	7 (35%)°	3 (15%)	0.1	3 (13%)	5 (22%)	4 (18%)	0.5
2-OH (n (%))	4 (20%)°	8 (40%)°	4 (20%)	**<0.05**	4 (18%)	7 (31%)	5 (22%)	0.3
S&E	30 [20–40]	40 [30–40]	0 [0–10]	**<0.05**	85 [67–90]	90 [70–90]	**0** [0–10]	0.1
HY	4 [4,5]	4	0 [0–1]	**<0.05**	2	2	/	1
Occurrence of AEs	6 patients (30%) = drowsiness; 1 patients (5%) = symptomatic OH				2 patients (9%) = drowsiness; 1 patients (4%) = hypertensive crisis			

Statistical significant results are in bold character.

The absent effect of L-dopa on fatigue in both populations is not surprising. Indeed, even if L-dopa has been proposed to induce a slower progression of fatigue compared with placebo [23], currently no treatment is considered effective for this NMS [24], and dopaminergic pathways seem to be only partially involved in the pathogenesis of fatigue in PD [23]. Even though the same seems true for anxiety, the rate of missing data among LSPD patients is too high to draw any firm conclusion. In fact, severity of anxiety moderately correlated with the motor improvement with L-dopa in both groups of patients. The acute effect of L-dopa on anxiety has been investigated in a few studies with small and heterogeneous samples of non-demented PD patients in intermediate/advanced stages. The findings suggest that L-dopa improves anxiety that fluctuates with L-dopa intake, whose magnitude is stronger in patients with motor "wearing-off" and that the fluctuation of anxiety correlates with the magnitude of motor response [25,26]. Accordingly, anxiety significantly improved after L-dopa in our advanced patients whose motor response to L-dopa was greater than in the LSPD group. The absent effect of L-dopa on anxiety among LSPD patients could be additionally explained due to a wider neurodegeneration of the locus coeruleus in the latest disease phase, which has been implicated in the pathogenesis of anxiety in PD [27,28]. Moreover, the lack of effect of L-dopa on anxiety in LSPD patients could also be related to the presence of an Alzheimer's disease-type pathology among LSPD patients, in which the presence of depression and anxiety may be mainly related to the presence of dementia [29,30]. Despite a lower L-dopa dose, the frequency of L-dopa-related AEs is slightly higher among LSPD patients than advanced ones. We may speculate that these AEs increase progressively with disease progression and the presence of dementia. Nevertheless, we did not find any correlation between frequency of AEs and disease duration, age, age at PD onset, PDD or disease severity of LSPD patients. The presence of these AEs, such as symptomatic OH, daytime sleepiness or hallucinations, frequently implies L-dopa dose reduction, making it even more difficult to manage PD in this late stage.

It could be interesting to investigate the acute and long-term effect of levodopa-carbidopa intestinal gel (LCIG) on NMS among LSPD patients. Indeed, some recent reports suggest an improvement of some NMS such as sleep/fatigue, pain, gastrointestinal and urinary symptoms, as assessed by the NMSS, during chronic treatment with LCIG [31–33]. Nevertheless the level of evidence for improvement of NMS is still considered low [[34] and no study has specifically addressed LSPD patients.

5.1. Study limitations

The sample size of the LSPD group was small, although these patients are very difficult to recruit [4]. The washout period for the STIM OFF condition in the advanced group was short, but many patients could not tolerate longer time without stimulation.

On the other hand, we could have investigated more NMS and also the several causes of pain in PD [21] and how they might respond differently to an L-dopa acute challenge. Importantly, our results concern the response of NMS to an acute intake of L-dopa and thus it may not indicate how these NMS respond to a chronic intake of L-dopa.

6. Conclusions

To the best of our knowledge, this is the first study that explores the response of non-motor variables to an acute L-dopa challenge in LSPD. Our exploratory study confirms the high severity and frequency of NMS among LSPD patients, and highlights the need for assessment tools adapted to these very disabled PD patients.

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Some NMS such as pain, fatigue and anxiety do not benefit from the acute action of a supra-threshold dose of L-dopa, which is in line with our recent findings for motor symptoms [10] and suggests an overall decrease of the effect of L-dopa with disease progression, at least its acute effect. Despite this, L-dopa retains the ability to induce AEs in LSPD patients; these AEs may possibly not occur if L-dopa dose is slowly increased. We acknowledge, however, that the benefit from an acute L-dopa challenge for pain, fatigue and anxiety in earlier stages of PD is not well established, in contrast to the amount of evidence of its effect on motor symptoms. Thus we can speculate that clinicians should not expect any gain from L-dopa dose increase for those NMS in LSPD patients. In fact, they should be cautious when trying to increase the dose of L-dopa, as frequent L-dopa-related AEs may occur, namely somnolence and arterial hypotension. They should indeed try to decrease L-dopa dose when facing troublesome daytime somnolence or arterial hypotension.

The expected increase in the prevalence of this orphan population, the limitation of current assessment scales and the apparent lack of response of certain NMS to L-dopa highlight the need for larger studies of LSPD in order to optimize the assessment of these patients and the treatment of NMS, which are a major source of disability in later PD stages.

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Speech and Voice Response to a Levodopa Challenge in Late-Stage Parkinson's Disease

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Background: Parkinson's disease (PD) patients are affected by hypokinetic dysarthria, characterized by hypophonia and dysprosody, which worsens with disease progression. Levodopa's (L-dopa) effect on quality of speech is inconclusive; no data are currently available for late-stage PD (LSPD).

Objective: To assess the modifications of speech and voice in LSPD following an acute L-dopa challenge.

Method: LSPD patients [Schwab and England score <50/Hoehn and Yahr stage >3 (MED ON)] performed several vocal tasks before and after an acute L-dopa challenge. The following was assessed: respiratory support for speech, voice quality, stability and variability, speech rate, and motor performance (MDS-UPDRS-III). All voice samples were recorded and analyzed by a speech and language therapist blinded to patients' therapeutic condition using *Praat* 5.1 software.

Results: 24/27 (14 men) LSPD patients succeeded in performing voice tasks. Median age and disease duration of patients were 79 [IQR: 71.5–81.7] and 14.5 [IQR: 11–15.7] years, respectively. In MED OFF, respiratory breath support and pitch break time of LSPD patients were worse than the normative values of non-parkinsonian. A correlation was found between disease duration and voice quality ($R = 0.51$; $p = 0.013$) and speech rate ($R = -0.55$; $p = 0.008$). L-Dopa significantly improved MDS-UPDRS-III score (20%), with no effect on speech as assessed by clinical rating scales and automated analysis.

Conclusion: Speech is severely affected in LSPD. Although L-dopa had some effect on motor performance, including axial signs, speech and voice did not improve. The applicability and efficacy of non-pharmacological treatment for speech impairment should be considered for speech disorder management in PD.

Keywords: Parkinson's disease, late stage, levodopa, speech, voice

INTRODUCTION

Parkinson's disease (PD) patients are classically affected by hypokinetic dysarthria, characterized by hypophonia and dysprosody, that worsens with disease progression due to breathing, phonation, and articulation dysfunction (1–3). Speech disorders affect nearly 90% of PD patients and have a negative impact on functional communication, which in turn contributes to decreased quality of life (4, 5). Symptoms vary from a soft and breathy voice that lacks modulation in volume (monoloudness) and fundamental frequency (monopitch or monotone) resulting in flat speech melody (dysprosody), with pitch breaks, lack of rhythm and pace of speech, number of pauses, reduced stress, and imprecision in consonant articulation, to a voice that is neither audible nor intelligible (6–9).

The effect of levodopa (L-dopa) on the quality of speech is inconclusive given that it is also influenced by each patient's speech profile. Some studies report on a slight improvement of intonation, vowel articulation, and speech intelligibility (10–13), while others show no significant effect (14, 15) as measured during an acute L-dopa challenge. Nevertheless, speech is generally considered to be an "L-dopa-resistant" axial motor symptom of PD (16). Axial impairment is preponderant among PD patients in the latest disease stage (17), although no data are currently available on the effect of L-dopa on speech among late-stage PD (LSPD) patients. The purpose of this study was to assess the clinical and active modifications of speech and voice after an acute L-dopa challenge in an LSPD population.

PATIENTS AND METHODS

Design and Recruitment

We performed a cross-sectional study in a consecutive sample of LSPD patients recruited during 12 months from the movement disorders outpatient clinic of a tertiary university hospital (Hospital Santa Maria, Lisbon, Portugal). PD was defined according to the UK Brain Bank criteria (18), whereas LSPD was defined as PD patients with either a Schwab and England score <50 (MED ON) or a Hoehn and Yahr stage (HY) >3 (MED ON) (19). The Local Ethics Committee approved the study. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Assessment of Patients

Late-stage PD patients were first assessed in the practically defined "MED OFF" condition and then 60–90 min after L-dopa intake in the best "MED ON" condition. For the L-dopa challenge, each patient took her/his usual morning L-dopa equivalent dose plus 50% (supramaximal dose = 150%). L-Dopa equivalent daily dose was calculated according to recognized standard conversions (20). Details of the L-dopa challenge have been previously reported (19).

The following parameters were assessed during both MED OFF and MED ON: (a) motor performance by means of the MDS-UPDRS part III (21); (b) severity of dyskinesias using the Modified Abnormal Involuntary Movement Scale (mAIMS); (c) respiratory support for speech (time duration of vowel/a/ prolongation); (d) voice quality [fundamental frequency (F_0)]; (e) voice stability (pitch break time and jitter); (f) voice variability [SD of speaking F_0 during sentences (Sentence F_0SD)]; and (g) speech rate (syllables/s). Each participant had to perform several vocal tasks that consisted of the following: (i) sustained phonation of the vowel/a/at a comfortable pitch and loudness and (ii) repeating an 8-word, 14-syllable standard statement/ declarative sentence, "A Maria comprou-me um mapa do papel branco" [translation: Mary bought me a map of white paper]; and (iii) reading 5 words and 5 sentences. Tasks were selected from the European Portuguese version of the Frenchay Dysarthria Assessment version 2 (22). However, due to the low level of cooperation of LSPD patients, we adopted an 8-word (14 syllables) declarative sentence (syntactically simple) that in European Portuguese is expected to have a low level of voice variability compared to complex sentences or text reading, which are normally used for this task.

Patients were seated and instructed by a neurologist to sustain the vowel/a/at a comfortable pitch and loudness as long as they could. A demonstration was made by the clinician before the patient performed each vocal task. There were no time limits for each participant and he/she was asked to repeat the task if the examiner was not fully satisfied with patient's performance.

All voice samples were recorded in a room in a home environment using a tabletop unidirectional microphone (Fame, MS-1800S) attached to a preamplifier (M-Audio Fast Track Pro, preamp, USB) and a desktop computer running *Audacity software version 2.1.2* (Free software Foundation Europe, Hamburg, Germany).

Two separate perceptual files were completed using *Audacity software* version 2.1.2 with all the stimuli presented at the same sound pressure levels and with a 500 ms silence between single words and sentences.

MDS-UPDRS parts II and IV were used to assess the impact of motor symptoms on activities of daily life and L-dopa-induced motor complications, respectively. PD with dementia was diagnosed according MDS Task Force recommendations (23).

Data Analysis

All voice samples were copied to a computer (down sampled to 24 kHz, 16 bits, mono), edited into individual files and screened for extraneous noise using *Audacity* by a speech language therapist with expertise in experimental phonetics and who was not involved in data gathering and was blind to the participants' demographics and clinical status.

Acoustically, the waveform, spectrogram, pitch, intensity, and the formants of each sustained vowel were visually observed using the *Praat* 5.1 software (24) downloaded from http://www.praat.org.

The vowel/a/mean and SD F_0 (Hz), jitter (local, %) and harmonic-to-noise-ratio (dB) were analyzed with a moving window with at least 1-s using voice report in the *Praat* software.

The following parameters were analyzed: (a) *Respiratory support for speech*. Duration (s) was measured as the total period between the onset and offset of each sustained vowel/a/and the breath(s) during speech in the sentence "A Maria comprou-me

um mapa de papel branco"; (b) *Voice (pitch) quality*. The average F_0 (Hz) was analyzed in all vowels in the two moments. Vowel/a/ was perceptually analyzed by a speech language therapist for pitch and loudness level along the production (mainly high or low); (c) *Voice (pitch) stability*. The assigned acoustic parameters were as follows: Pitch breaks (no pitch contour) time (seconds) and jitter (local, %). Vowel/a/was perceptually analyzed by considering the pitch and loudness stability (maintained, increased, decreased or uncontrolled); (d) *Voice variability*. Variability was considered as speech F_0SD in hertz in the sentence (Sentence F_0SD). At baseline (MED OFF) the F_0SD (Hz) was also analyzed; and (e) *Speech rate*. Speech rate of the sentence "A Maria comprou-me um mapa de papel branco" [Mary bought me a map of white paper], total number of orthographic syllables divided by total time duration (including pauses).

Statistical Analysis

Descriptive statistics of demographic, clinical, and therapeutic data were provided for continuous (median and interquartile range [IQR, 25th–75th percentile]) and categorical (count and percentage) variables.

Voice and speech characteristics at baseline (MED OFF) of LSPD patients, considering men and women separately, were compared to the available normal values of healthy age-matched subjects, although no statistical analyses were performed.

The acute effect of L-dopa on voice and speech was calculated by comparing the median duration of the vowel/a/, average F_0, pitch breaks duration, jitter, SF_0SD, and speech rate between MED OFF versus MED ON conditions. Comparisons were made using the Wilcoxon's signed-rank test.

Spearman's rank correlation coefficient was used to assess the association between: (a) respiratory support for speech, voice quality, voice stability, voice variability, speech and disease duration, and motor impairment (MDS-UPDRS-III)/axial motor impairment (sum of items 3.1, 3.10–3.12 of the MDS-UPDRS-III); (b) speech rate and freezing (item 3.11 of the MDS-UPDRS-III).

Two group comparisons (women versus men) were performed using the Mann–Whitney *U*-test.

Reliability of Analyses

To evaluate test–retest reliability of acoustic measurements the sustained vowel/a/for an average F_0 was run twice. A satisfying test–retest reliability was found ($R = 0.722$, $p < 0.001$, Pearson test), only one single-speech-task cycle was performed for the definite acoustic measurements.

A p value < 0.05 was considered significant. The analysis of the results was carried out by means of SPSS 21.0 (SPSS, Chicago, IL, USA).

RESULTS

Clinical Data

Twenty-seven LSPD patients were recruited for speech and voice analyses. Three were excluded due to their inability to perform the required tasks (one anarthric patient and two due to severe dementia). Demographic and clinical data of the 24 LSPD patients are detailed in **Table 1**.

There were no differences in demographic or clinical variables between men and women (**Table 1**). Indeed, they presented similar MDS-UPDRS II–III–IV scores, axial signs score, SE and HY stages, although women had a slightly, but not statistically significant, worse HY stage, and more men were demented although not statistically significant (**Table 1**).

Baseline (MED OFF) Voice and Speech Characteristics

No differences were found between men and women for breath support and voice stability at baseline (MED OFF) (**Table 2**). Voice quality differed between men and women at baseline, although this difference has been noticed in vocally healthy subjects (gender effect) and the values were also similar to vocally healthy subjects (25) (**Table 2**). Values of respiratory breath support

TABLE 1 | Values are presented as median [IQR, 25th–75th percentile] if no otherwise specified.

Patients data	LSPD ($n = 24$)	LSPD Male ($n = 14$)	LSPD Female ($n = 10$)	p-Value
Age (years)	79 [71.5–81.7]	77.5 [70.7–81.2]	79 [73.5–85]	ns
Age at disease onset (years)	64.5 [54.5–69.5]	62.5 [55–67]	65 [51.5–71.5]	ns
Disease duration	14.5 [11–15.7]	13.5 [8.7–17]	15 [11.7–17.2]	ns
Education (years)	4 [4–11]	4 [4–12]	5 [4–10.5]	ns
S&E (ON/OFF)	40/35 [40–40.7/22.5–40]	40/30 [40–40/40–40]	40/30 [27–50/17.5–50]	ns
HY (ON/OFF)	4 [2–4]/4 [2–4.75]	3 [2–4]/3 [2–4]	4 [4–5]/4 [4–5]	ns
PDD [*n* (%)]	14 (58%)	10 (71%)	4 (40%)	ns
MMSE	22.5 [21.2–25]	22.5 [22–24.2]	22.5 [16–27.2]	ns
MMSE (demented/non-demented)	22 [17–23.7]/25 [23–26.7]	22 [21.7–24.2]/23 [22.2–25.2]	17 [13–19.5]/27 [25–28.5]	
LEDD (mg)	1,037 [902–1,272]	1,100 [990–1,303]	905 [742–1,257]	ns
MDS-UPDRS-II	31 [27–38]	32 [29.2–38.5]	30 [20.5–38]	ns
MDS-UPDRS-III (MED ON/MED OFF)	50 [40–54]/64 [52–77]	50 [42.5–55.2]/61 [53–76]	50 [37.5–62.5]/64 [48–79.5]	ns
Axial sign (MED ON/MED OFF)	8 [6–13]/10 [7–13]	8 [6–13]/10 [7–13.2]	8 [6.5–12]/10 [7–13.5]	ns
MDS-UPDRS-IV	4 [2–9.5]	5 [2–8.5]	4 [0–11.2]	ns

LEDD, L-dopa equivalent daily dose; PDD, Parkinson's disease with dementia; MMSE, mini mental state examination; S&E, Schwab and England score; HY, Hoehn and Yahr stage; ns, non-significant; LSPD, late-stage PD.
p-Value is the results for male versus female scores' comparison.

TABLE 2 | Values for late-stage PD patients are presented as median [IQR, 25th–75th percentile].

	Parkinson's disease patients ($N = 24$)		Normal value	
Respiratory support for speech				
Vowel duration (s)	5.8 [4.4–11.5.8]		22.97 (1.1)[b]	
Voice stability				
Pitch break time (s)	1.24 [0.2–2.6.1]		NA[a]	
Jitter (%)	0.8 [0.5–1.1]		≤0.5–1%	
Voice variability				
Sentence F_0SD (Hz)	2.4 [1.6–4]		2–4 Hz	
Voice quality (Hz)	**Male ($N = 14$)**	**Female ($N = 10$)**	**Male**	**Female**
F_0	125 [104–152]	202 [160–226.8]	128 (36)[c]	198 (44)[c]

Values for healthy subjects are presented as mean (SD), as reported in literature (25–28).

F_0, fundamental frequency; Sentence F_0SD, SD of speaking F_0 during sentences.

[a]Not available (healthy voices should have no trouble in maintaining voicing during a sustained vowel. Thus is 0% of voice breaks. No standard values are available).

[b]Normal value for vowel duration is referred to a healthy population aged between 71 and 80 years old.

[c]Normal value for voice quality is referred to a healthy population aged between 55 and 80 years old.

(26) and pitch break time (24) of LSPD patients appeared worse when compared to the normal values of healthy age-matched subjects, stratified for gender (**Table 2**). Mean jitter values were in the normal range (**Table 2**), although results were borderline for men and SD showed a tendency for higher values (27). In contrast, F_0SD (28) was in the normal range (**Table 2**). However, this result was partially expected as we use a very syntactically simple sentence.

A positive moderate correlation was found between disease duration and voice quality ($R = 0.51$; $p = 0.013$) and a negative one with speech rate ($R = -0.55$; $p = 0.008$). Motor impairment (MDS-UPDRS-III) had a moderate significant correlation with respiratory support for speech ($R = -0.43$; $p = 0.045$) and pitch break time ($R = -0.565$; $p = 0.006$). No correlations were found between voice and speech features and axial motor impairment, neither between speech rate and freezing. When analyzing by gender (men and women separately) such correlations were partially maintained: (a) voice quality and disease duration: men ($R = 0.5$; $p = 0.079$) and women ($R = 0.36$; $p = 0.2$); (b) speech rate and disease duration: men ($R = -0.7$; $p = 0.003$) and women ($R = -0.2$; $p = 0.5$); (c) respiratory support for speech and MDS-UPDRS-III: men ($R = 0.64$; $p = 0.017$) and women ($R = -0.7$; $p = 0.029$).

L-Dopa Acute Challenge Test

No differences between men and women were found when comparing motor, voice, and speech variables during both MED OFF and MED ON, except for voice quality (F_0), as was expected (see **Table 2** for voice characteristics of healthy subjects). Thus, further analyses were carried out by taking into consideration the whole LSPD sample and not stratifying by gender.

Motor Response

The median L-dopa dose for the test was 375 mg [IQR: 277–375]. The median MDS-UPDRS-III score was 64 [IQR: 52–77] in MED OFF and 50 [IQR: 40–54] in MED ON, with a significant median improvement of 20% [IQR: 11.5–32%] ($p < 0.001$) (**Table 3**). Sub-analysis of MDS-UPDRS-III scores for axial signs showed a significant median improvement after L-dopa intake for all the subitems, except speech (**Table 3**). 3 patients (12.5%) had mild dystonic dyskinesias in MED OFF, while 12 (50%) presented slight-moderate choreic dyskinesias in MED ON.

TABLE 3 | Values are presented as median [IQR, 25th–75th percentile].

	LSPD patients ($N = 24$)		
	MED OFF	**MED ON**	***p*-Value**
MDS-UPDRS-III	64 [52–77]	50 [40–54]	**<0.001**
Speech	2 [1–3]	2 [1–3]	0.83
Freezing of gait	3 [1–4]	2 [0–3]	**<0.05 (0.01)**
Postural stability	3 [2–4]	3 [2–3]	**<0.05 (0.014)**
Gait	3 [2–4]	3 [2–3]	**<0.05 (0.01)**
Axial signs	10 [7–13]	8 [6–13]	**<0.05 (0.01)**
HY	4 [2–4.75]	4 [2–4]	0.7
mAIMS	0	1 [0–6.75]	**0.04**
Voice respiratory support for speech			
Vowel duration (s)	5.8 [4.4–11.5]	7 [3.6–10.6]	0.6
Voice stability			
Pitch break time	1.2 [0.2–2.6]	0.8 [0.07–2.5]	0.9
Jitter	0.8 [0.5–1.1]	0.7 [0.4–1]	0.5
Voice quality			
F_0	154 [123–209]	162 [147–203]	0.2
Voice variability			
Sentence F_0SD	31 [19–51]	29 [20–40]	0.5
Speech rate	5 [3.6–5.6]	5 [4.2–5.7]	0.2

Statistical significant results are in bold. Axial signs: sum of item 3.1, 3.10–3.12 of the MDS-UPDRS-III. p-Value is the results of MED OFF versus MED ON scores.

mAIMS, Modified Abnormal Involuntary Movement Scale; F_0, fundamental frequency; Sentence F_0SD, SD of speaking F_0 during sentences; LSPD, late-stage PD; HY, Hoehn and Yahr stage.

Voice and Speech Response

None of voice and speech variables changed significantly after L-dopa intake (**Table 3**).

Equally, separate analysis of non-demented and demented patients showed no modification of speech and voice variables following L-dopa intake.

DISCUSSION

The purpose of this study was to explore the L-dopa response of speech in the late stage of PD. In order to do this a population of LSPD patients underwent an L-dopa challenge while performing specific vocal tasks during both MED OFF and MED ON conditions. No effect of L-dopa was found on speech and voice by means of both automated analysis and clinical evaluation, although patients had a moderate positive motor response, even present for some axial signs, with the exception of speech. Such a discrepancy in L-dopa responsiveness between speech and other axial signs has been reported only in one previous speech study in advanced PD patients (14) and suggests that speech together with balance and postural problems could be listed among L-dopa resistant axial sign appearing with disease progression.

Despite not performing a case-controlled study, by comparing MED OFF speech and voice characteristics of our patients with normative values of the general population we found a severe impairment of respiratory support for speech and voice stability, as already reported elsewhere (6, 12). We chose to make this comparison in the MED OFF condition because it more accurately reflects the parkinsonian state of patients. Rigidity associated with PD can often lead to disruption of respiratory processes which serve to generate air pressure for speech (10). Respiratory support for speech may be measured through vowel prolongation, and a decrease by an average of fifty percent in vowel prolongation has been reported for PD patients when compared to normal healthy speakers (10). Among our LSPD patients, vowel prolongation was more affected, even in the absence of dyskinesias that can affect respiratory control (11). Equally, voice stability, i.e., ability to maintain a consistent voice during a stable/sustained vowel with laryngeal muscle effort, is impaired in MED OFF, as shown by an increase in pitch break time and the tendency for jitter increment. Moreover, a tendency for worsening voice quality and speech rate was highlighted with disease duration. Voice quality and voice variability values in MED OFF were in the normal range although the most plausible cause for this finding is methodological, which might have resulted in falsely normal values for voice quality and variability: we have chosen a declarative sentence for voice variability analysis that is syntactically too simple to capture this feature; equally, we assessed voice quality using mean F_0 instead of F_0SD which is usually more appropriate but not possible to analyze in our patients due to the technical quality of the recordings. Interestingly, no correlations were found between speech rate and freezing. These data are apparently in contrast with the recent findings of Ricciardi and colleagues that showed lower scores in the articulation, intelligibility, rate/prosody section of the Dysarthria Profile in PD patients with freezing of gait (FOG), as assessed by the New FOG questionnaire, if compared to PD patients without FOG (29). However, in our study, different methodological measures have been adopted in order to assess both speech rate and FOG. Moreover, Ricciardi and colleagues included younger PD patients, belonging to several HY stages, thus a more heterogeneous PD sample, scarcely comparable to our LSPD patients.

Our sample of LSPD patients still presented moderately good motor response to L-dopa (20% of the MDS-UPDRS-III) when compared to our previous report (19), and the frequency of dementia was slightly lower (52%) (19). The exclusion of patients who could not speak at all or who could not properly understand the tasks would have surely created bias. Thus, our sample may represent a subset of LSPD patients who present a slightly better clinical state compared to other reports (30, 31). Nevertheless, even if an influence of dyskinesias on speech performance cannot be excluded (11), speech showed no improvement after L-dopa intake, whether it was measured clinically or with automated analysis that explored the respiratory support for speech (vowel duration), voice stability, variability and quality, and speech rate. De Letter et al. evaluated respiratory features among 25 non-demented PD patients during an L-dopa challenge and reported a slight improvement of sustained vowel phonation (11). However, due to the clinical differences with our sample, i.e., older patients with longer disease duration and worse L-dopa response, these results may not be comparable with those published by De Letter et al. Concerning voice stability and variability, if we assume that hypokinesia of the voice apparatus is the major pathological mechanism of monopitch speech in PD (32, 33), F_0SD should improve after L-dopa intake and should decline further during the disease course. However, data on voice stability/variability improvement after L-dopa are inconsistent, and previous reports have also failed to show a response of F_0SD or jitter to dopaminergic therapy (12, 15, 34). This finding may be related to the usual worse response of axial muscles to L-dopa.

A lack of improvement in speech quality (F_0) and speech rate after L-dopa or apomorphine has already been described in earlier PD stages (12, 14, 15, 35). We report similar data in LSPD patients, although we have to consider that our patients did not present with a severe impairment of voice quality in MED OFF. Thus, an improvement would not be expected. A slight improvement of speech rate after L-dopa intake has been found in only nine PD patients with optimal L-dopa responsiveness and a non-severe impairment of speech at baseline, as assessed by the UPDRS-III (34). However, Ho et al. concomitantly reported on a decay of rate improvement during the speech testing tasks (34). Thus, it is likely that improvement in speech rate is not maintained during the tasks.

Several factors can contribute to the lack of speech and voice responsiveness to L-dopa in PD patients, especially in the late disease stage.

Speech production is essentially a series of skilled motor gestures that require upstream central coordination mediated by cerebral networks for speech production. Indeed, the globus pallidus (GP) produces a phasic burst of activity that triggers the supplementary motor area neural discharge, allowing cortical motor set for movement preparation and subsequent execution (34). In PD, the impairment of GP activity alters those mechanisms, resulting in diminished movement amplitude and impairment of movement sequencing. Such a process affects speech production as well as body movement, and a correlation between speech hypophonia/speech intensity and severity of body bradykinesia has been suggested (34). L-Dopa has been shown to have an effect on preparatory motor set, resulting in hypokinesia improvement, but failed to affect movement sequencing (36). Likewise, concerning speech, while still controversial, a few studies have reported on a slight L-dopa positive effect on loudness (speech intensity), intonation (speech variability), and speech rate (12, 34) at least in early-advanced PD stages. Conversely, speech stability and variability seem to be definitively impervious to dopaminergic therapy (9, 12). Interestingly, and contrary to previous suggestions, we did not find neither an improvement of speech intensity or rate with L-dopa nor a correlation between speech and voice severity and motor symptoms that still respond to L-dopa, namely, bradykinesia and rigidity. These findings may support a non-dopaminergic involvement in speech neurocircuitry as already supposed in earlier disease stages (35), and this is even more likely in LSPD (37). Alternatively, a higher dose of L-dopa could be needed to improve speech, as is often the case with gait dysfunction. The usual absence of significant rigidity in late-stage patients (19, 31) may also have contributed

to the lack of correlation between speech intensity and motor impairment. Furthermore, we have to consider that a loss of striatal responsiveness is related to disease progression and is likely responsible for a decrease or loss of clinical response to dopaminergic therapy of several motor symptoms (19), which also probably affects speech responsiveness. Finally, motor speech production also depends on the appropriate function of peripheral nervous system (7). Dysfunction of speech articulation may also be partly attributed to muscular denervation and atrophy, resulting in respiratory muscles impairment whose function does not improve with L-dopa as recently shown in a sample of PD patients in HY 2–4 (38). Such muscle impairment is presumably even more severe among older PD patients who have a worse motor status as our sample.

Our findings highlight the need for alternative non-dopaminergic/non-pharmacologic treatments to improve communication of LSPD patients. For instance, the Lee Silverman Voice Treatment has shown some efficacy in the treatment of voice and speech problems of PD patients (7). However, its applicability to LSPD patients should be verified due to the level of collaboration that it requires and the degree of disability of those patients.

Study Limitations

Some limitations of our study must be highlighted. Due to the clinical disability of LSPD patients, recordings were performed in a home environment and not in a laboratory setting. This implied accepting samples varying in context, over different time periods, and recorded in non-standard environments. Nevertheless, the quality and reliability of the recordings were evaluated by a speech language therapist. Patients' disabilities can also have influenced choice of tasks. For instance, we selected a simple task for voice variability assessment, which was probably not sensitive enough to detect L-dopa effect in voice/intonation variability, or voice variability defect at baseline. Finally, clinical assessment of patients was not blinded. However, there was concordance between clinical and automated assessments of speech.

Conclusion

To the best of our knowledge, this is the first report on L-dopa response of speech and voice in a sample of LSPD patients by means of both a clinical rating scale and automated analysis. Speech is severely affected among LSPD patients, as already reported for PD patients in earlier disease stages (1, 4).

Although L-dopa still had some effect on motor performance, including some axial signs, we found no improvement in speech and voice. Clinical management and research should consider the applicability of non-pharmacological treatments for speech and voice impairment among LSPD patients.

ETHICS STATEMENT

The Local Ethics Committee of the "Centro Hospitalar Lisboa Norte, Lisbon, Portugal," approved the study. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

MF and IG: substantial contributions to the conception and design of the work, drafting the work; final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; RC and MC: substantial contributions to the conception and design of the work; final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; LG and MR: substantial contributions to the interpretation of data for the work; revising the work critically for important intellectual content; and final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; CG: substantial contributions to the conception or design of the work, revising it critically for important intellectual content; final approval of the version to be published and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; DA and NG: substantial contribution to the analysis of data, revising the work critically for intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; AA and JF: substantial contributions to the interpretation of data for the work, revising the work critically for important intellectual content, final approval of the version to be published, and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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