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Final dissertation

Pathological Phenotypes in Parkinson's Disease: From Biomarkers to Treatments

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CONTENTS

PART 1

Introduction

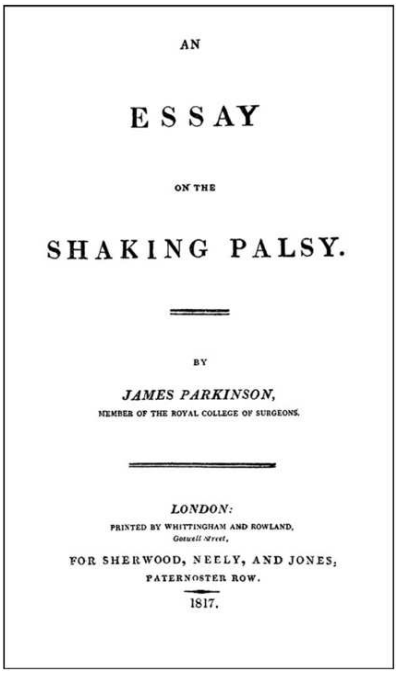
Parkinson's disease is a neurodegenerative disease that affects around 3% of the population older than 80 years old and, at the moment, it is considered the most frequent cause of parkinsonism (Li & Le, 2019). There are a variety of symptoms that Parkinson's disease patients can show, with motor and non-motor symptoms being involved. The diagnosis of PD is made with clinical observation, usually of the cardinal motor features, which are tremor, rigidity, bradykinesia (slowness of movement), and postural instability, but there are other motor symptoms that PD patients can present with (Beitz, 2014; Jankovic, 2007; Li & Le, 2019). The non-motor symptoms that Parkinson's disease patients usually show are neuropsychiatric symptoms, such as depression, anxiety, impulse control disorder, and cognitive impairment; sensory dysfunction; disturbance of the sleep-wake cycle; autonomic dysfunction; and gastro-intestinal symptoms (Chaudhuri et al.,2012; Poewe, 2008). All those symptoms, motor and non-motor, interfere with the patient's function and activities of daily living (Li & Le, 2019). The cause(s) of Parkinson's disease is yet to be established, but it could be related to some environmental factors and genetic mutations, with alpha-synuclein and Lewy pathology playing an important role in the pathology of the disease (Kalia & Lang, 2015; Movement disorder).

Currently, one of the most researched topics in Parkinson's disease is the detection of a biomarker, which is a measurable biological sign of a disease or health condition (Koníčková et al., 2022; Li & Le, 2019). The discovery of an accurate and strong biomarker for PD is of extreme importance, as it would lead to the possibility of recognizing Parkinson's disease early, even before the appearance of the motor symptoms, and would assist in the differential diagnosis (Li & Le, 2019; Youssef et al., 2023). Some potential biomarkers being studied for PD are genetic, CSF and blood, neuroimaging, microbiome, and inflammation related biomarkers (Sauerbier et al., 2016; Stocchi et al., 2024).

What is Parkinson's Disease:

Parkinson's Disease (PD) is the second most common neurodegenerative disease today, following Alzheimer's Disease (AD). It is the fastest-growing neurological disorder globally, affecting 1% of the population over the age of 60 years old and 3% over the age of 80, being slightly more frequent in men than in women (Beitz, 2014; Li & Le, 2019; Stocchi et al., 2024). James Parkinson was the first to describe Parkinson's disease as a clinical entity in 1817 in *An Essay on the Shaking Palsy* (figure 1) as an "involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace: the senses and intellects being uninjured", he also described the presence of constipation, cognitive dysfunction, and sleep disorders (Antonini et al., 2023; Goetz, 2011). Some decades later, Jean-Martin Charcot rejected the first designation of the illness as a shaking palsy as he noted that PD patients were not markedly weak and didn't necessarily have tremors. He then suggested the name Parkinson's disease (Goetz, 2011).

Figure 1. *An essay on the Shaking Palsy.*



AN

ESSAY

ON THE

SHAKING PALSY.

BY

JAMES PARKINSON,

MEMBER OF THE ROYAL COLLEGE OF SURGEONS.

LONDON:

PRINTED BY WHITTINGHAM AND ROWLAND,
Goswell Street,

FOR SHERWOOD, NEELY, AND JONES,
PATERNOSTER ROW.

1817.

Note. James Parkinson first described Parkinson's disease in "an essay on the shaking palsy". From "The History of Parkinson's Disease: early clinical descriptions and neurological

therapies", by Goetz, C. G., 2011, *Cold Spring Harbor Perspectives in Medicine*, *1*(1), a008862. Figure 1 (https://doi.org/10.1101/cshperspect.a008862). CC BY.

Today, Parkinson's disease is considered a chronic and progressive neurodegenerative disorder and the most common primary cause of Parkinsonism (Li & Le, 2019). It is characterized by the loss of dopaminergic neurons within the pars compacta of the substantia nigra, which leads to various motor (e.g., bradykinesia, tremor, rigidity, gait disturbance) and non-motor symptoms (e.g., neuropsychiatric symptoms, sleep disorders, autonomic symptoms, pain) that can affect the patient's activities of daily living and function (Li & Le, 2019). Nowadays, Parkinson's Disease is being thought of as a systemic multi-organ disorder, that involves other pathways and neurotransmitters instead of only dopamine. This idea is related to the identification of pathological hallmarks (alpha-synuclein) in many tissues in the body and not just in the central nervous system (Antonini et al., 2023).

The cause(s) of Parkinson's has not yet been established. Still, it is believed to be related to some genetic changes (e.g., LRRK2, SNCA, PINK1, DJ) and environmental factors (e.g., pesticides, head injury, area of residency, occupation) (Delamarre et al., 2017; *Parkinson's Foundation*).

Alpha-synuclein (α-syn) oligomers and Lewy pathology have an important role in Parkinson's disease pathology. When misfolded, α-syn can aggregate into oligomers, forming Lewy bodies (abnormal protein aggregates inside neurons) and Lewy neurites (abnormal neural processes), which can contribute to the degeneration of dopaminergic neurons (Kalia & Lang, 2015; Yamashita et al., 2023).

The diagnosis of Parkinson's disease is still made based on clinical observations, mainly of its motor symptoms: bradykinesia, rigidity, and resting tremor. Some neuroimaging techniques, such as MRI or CT scan, can help and support the clinical evaluation, but the diagnosis can only be confirmed post-mortem (*Movement disorder*; Sobel, 2015).

Neuropathological hallmarks of Parkinson's Disease

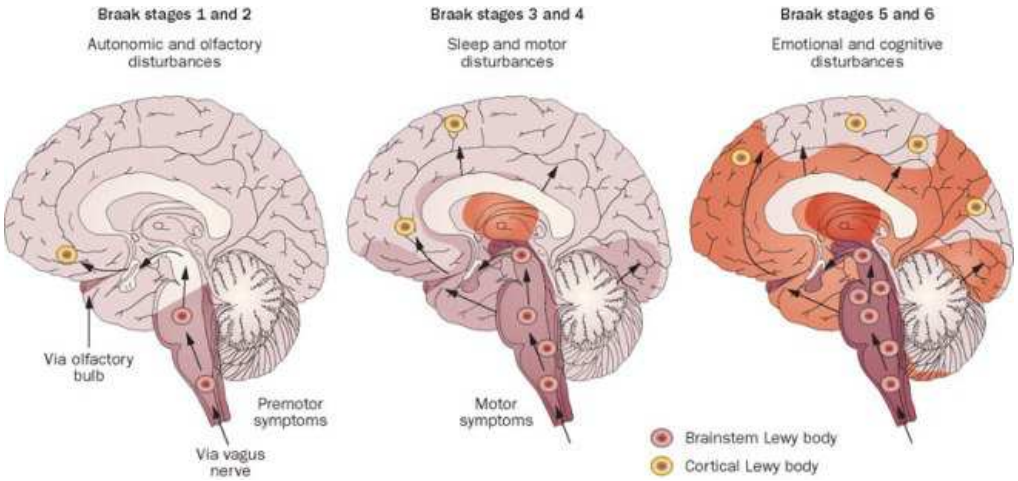
Traditionally, clinical Parkinson's disease has been linked to the degeneration of dopaminergic neurons of the Substantia Nigra (SN), together with Lewy bodies. In 1938, a post-mortem study made by Hassler showed that the putamen, globus pallidus, and caudate were not altered in Parkinson's, while in the ventral part of the Substantia Nigra dense clusters of neurons were severely impacted with the remaining cells usually containing Lewy bodies. During the 1950s, it was discovered the dopaminergic nature of the nigrostriatal neurons, which resulted in the recognition of the role of dopamine. In 1997, it was found that familial PD is caused by a mutation of the SNCA gene. Later, PD was termed an α-synucleinopathy, being included in the class of proteinopathies that have as common properties aggregated α-synuclein (Sobel, 2015). Today, it is growing the amount of research that supports viewing Parkinson's as a systemic condition, in which various neurotransmitters and organs play a role (Antonini et al., 2023).

Evidence suggests that in Parkinson's pathogenesis, the formation of α-synuclein oligomers, Lewy bodies, and fibrils have a crucial role, but what triggers this process is still not known (Stocchi et al., 2024). PD seems to arise from the complex interplay of abnormal α-synuclein aggregation, dysfunction of mitochondria, lysosomes or vesicle transport, synaptic transport issues, and neuroinflammation. All its mechanisms lead to an acceleration in neuronal death of primarily dopaminergic neurons, but there are many other non-motor and motor circuits involved in the neuropathology of Parkinson's disease (Bloem et al., 2021).

According to Braak et al., 2003, there is a pattern of deposition of α-synuclein during the progression of PD, which he described in six progressive stages (figure 2). In the 1st and 2nd stages, the patient is clinically presymptomatic and there are Lewy bodies present in the olfactory regions and in the lower brainstem. In stages 3 and 4, we can observe clinical symptoms of PD, such as tremor, rigidity, and bradykinesia, and the Lewy bodies are present in the midbrain, more specifically in the substantia nigra pars compacta (stage 3), in the forebrain, and in cortical areas (stages 4). During the last two stages, 5 and 6, we can observe

neuropsychiatric symptoms and cognitive impairment, and there is a substantial loss of dopaminergic neurons in the midbrain, with α-synuclein deposition in higher-order cortical association areas (Sobel, 2015).

Figure 2. *The Braak stages of PD*.

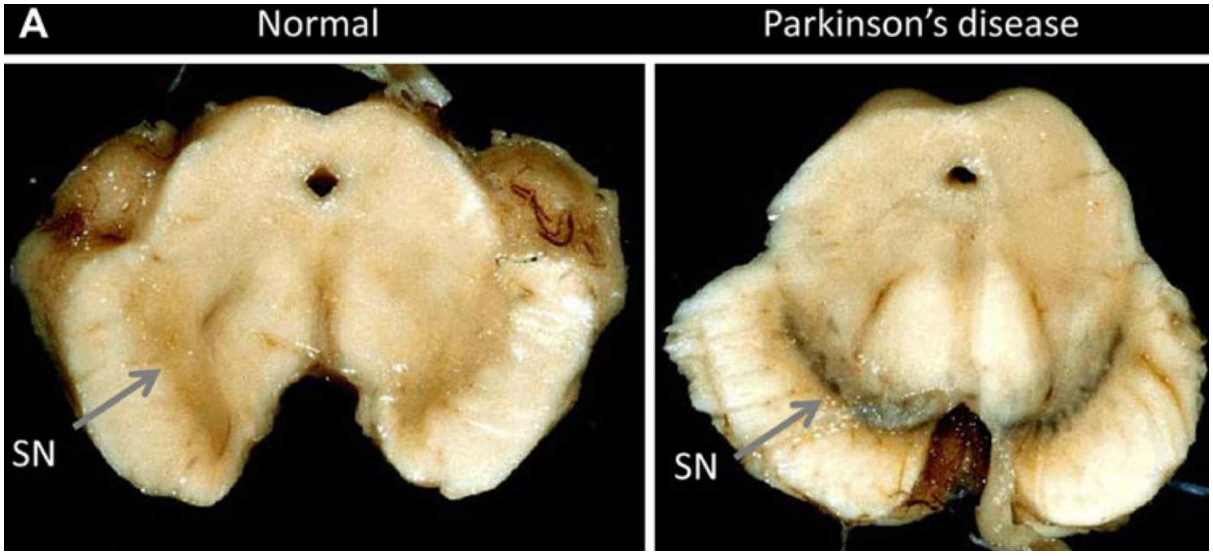


From "A gut feeling", by Simon, 2015, *The science of Parkinson's* (https://scienceofparkinsons.com/2015/09/09/september-7-13th-2015/). CC BY.

The main neuropathological hallmark of Parkinson's disease is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta, together with Lewy bodies that are mainly composed of α-synuclein (Choong & Mochizuki, 2022). The Substantia nigra is a dark structure, caused by elevated levels of neuromelanin that the dopaminergic neurons have, found in the midbrain. The most important role that the substantia nigra has is the production of dopamine, which is a neurotransmitter crucial in movement and motor control, emotional limbic activity, as well as cognitive executive functions (Grujicic, R., 2023). In Parkinson's disease, there is a loss of those dopaminergic neurons (figure 3) and, when around 40 to 60% of those neurons are lost, PD patients show the cardinal motor features (bradykinesia, tremor, postural instability, and rigidity) of the disease (Beitz, 2014). As previously mentioned, Lewy bodies are composed of α-synuclein, which is a synaptic protein that can be found in the neocortex, hippocampus, substantia nigra, thalamus, and cerebellum. In Parkinson's disease, α-synuclein is found in its monomeric form which misfolds and

aggregates into oligomers, having a prion-like spread. As the disease progresses, the accumulation of α-synuclein becomes more widespread throughout the brain (Chen et al., 2022; Stocchi et al, 2024).

Figure 3. *Example of substantia nigra of a Parkinson's disease patient and a normal subject.*



Note. On the right side, we have a normal and healthy substantia nigra, with dopaminergic neurons that are represented by the two dark lines. On the left side, we have a representation of the substantia nigra of a Parkinson's patient, in which we can see the loss of dopaminergic neurons. From "Biomarkers for prediction and targeted prevention of Alzheimer's and Parkinson's diseases: evaluation of drug clinical efficacy", by Mandel, S. A., Morelli, M., Halperin, I., & Korczyn, A. D., 2010, *The EPMA Journal*, *1*(2), 273–292, Figure 2 (https://doi.org/10.1007/s13167-010-0036-z). CC BY.

Physiopathology of Parkinson's Disease

Motor Symptoms

The cardinal motor features of Parkinson's Disease are bradykinesia, tremors, rigidity, and postural instability. They usually occur when around 40 to 60% of striatal dopamine nerve terminals are lost (Antonini et al., 2023). Those symptoms are used clinically for a diagnosis

of PD with their presence and specific presentation helping to differentiate Parkinson's Disease from other Parkinsonian disorders (Jankovic, 2007).

Bradykinesia is a term derived from the Greek used to refer to the slowness (brady) of movement (kinesis), which is one of the main motor symptoms of Parkinson's Disease (Bologna et al., 2019). It can also be present in atypical parkinsonism or other disorders, such as depression. Bradykinesia is thought to result from basal ganglia dysfunction, a failure of its output to the primary cortex, leading to difficulties in planning, performing sequential and simultaneous tasks, and initiating and executing movements (Bologna et al., 2019; Jankovic, 2007). Initially, the patient often presents with slow movements and reaction times, while also showing slowness while performing everyday activities. Bradykinesia can also interfere with various aspects of daily living, such as speech (hypophonia), loss of facial expression (hypomimia), decreased blinking, handwriting (micrographia), and dressing (Gasser & Wichmann, 2023; Jankovic, 2007). Usually, patients' movements (sitting down and walking) occur *en bloc*, which means the movement is made as a unit rather than into segments (Gasser & Wichmann, 2023). To assess bradykinesia, the patient is asked to perform rapid and repetitive tasks, alternating hand movements such as tapping the index finger and thumb together and/or rotating the palms up and down, and heel taps such as tapping the foot up and down, and is observed during those tasks if the patient shows a decrease in speed or amplitude of the movements (Hernández, 2023; Jankovic, 2007). Today, the treatment for bradykinesia can include the increase of dopamine levels in Parkinson's patients (Hernández, 2023).

Tremor can be explained as an involuntary, rhythmic, oscillatory movement of a part of the body (Movement Disorder). It is one of the first motor symptoms of Parkinson's Disease and it affects approximately 75% of the patients (Dirkx & Bologna, 2022). Initially, resting tremor is unilateral, meaning it affects only one side of the body, but as the disease advances, it can affect both sides (Janice, 2014). Tremors in PD can be manifested in two different manners, as a resting tremor (occurs when the body is relaxed and still), and as an action tremor (occurs when the patient is performing a movement), with resting tremor usually being the most

frequent and happening at a frequency between 4 and 6Hz (Dirkx & Bologna, 2022). The most frequent type of tremor Parkinson's patients have is the pill-rolling tremor at rest, which looks like the patient is trying to roll a pill between their thumb and index finger (Abusrair et al., 2022). Rest tremors can also affect the chin, lips, jaw, and legs of the patients, but is rare that it affects the voice, neck, and head (Jankovic, 2007).

Rigidity can be described as an increase in resistance to muscle movement that is experienced by the individual as a stiff muscle tone (Baradaran et al., 2013). It affects almost all patients with Parkinson's disease, contributing to feelings of discomfort and pain. Rigidity in PD can be characterized as "cogwheel rigidity", which is a jerky movement similar to cogwheels moving together (Jankovic, 2007).

Postural instability is the loss of ability to maintain an upright posture. It can be the most disabling motor symptom, as the patient is unstable and unbalanced when standing or trying to move around, leading to an increased risk of falls (Beitz, 2014; *Khan Academy*). It is usually developed later in the disease course. The patient can be assessed with the "pull test", in which the doctor pulls the patient backward or forward by the shoulder and then grades the patient's response (Tan et al., 2018).

The motor symptoms explained above are not the only motor manifestations of Parkinson's disease. We can have other motor symptoms present, such as hypomimia (masked face), freezing of gait (inability to move), dysphagia (difficulty swallowing), and dysarthria (speech disorder) (Beitz, 2014; Jankovic, 2007).

Non-motor symptoms

Parkinson's disease is considered a movement disorder. However, there are still some non-motor symptoms present in the clinical manifestation, such as neuropsychiatric symptoms (e.g., depression, anxiety, impulse control disorders, cognitive impairment), sensory dysfunction (e.g., hyposmia, pain), disturbance of sleep-wake cycle (e.g., insomnia, rapid eye movement sleep behavior disorder), autonomic dysfunction (e.g., orthostatic hypotension,

urinary urgency), and gastro-intestinal symptoms (e.g., constipation, dysmotility) (Chaudhuri et al.,2012; Poewe, 2008).

These non-motor symptoms will be present in most patients, and some of them can even appear before the occurrence of the first motor symptoms (Poewe, 2008). According to some available evidence, non-motor symptoms seem to have a bigger impact on the patient's and their caregiver's quality of life when compared with motor symptoms (Chaudhuri et al., 2012). Depression seems to affect around 45% of individuals with Parkinson's disease. The patient often presents with sadness, feelings of guilt, and a lack of self-esteem. Because its clinical manifestations (e.g., slowness, sleep disturbances, decreased concentration) are similar to some of Parkinson's symptoms, it is often difficult to diagnose, leading to many untreated patients (Campagnolo et al., 2023; Chaudhuri et al., 2012). Often coexisting with depression, anxiety affects between 25 to 40% of patients. It can appear as generalized anxiety disorder (GAD), phobias, or panic attacks. Sometimes, it can be an early sign of fluctuations and/ or therapeutic offs (Campagnolo et al., 2023; Chaudhuri et al., 2012). Another neuropsychiatric symptom of PD is impulse control disorder. Patients may exhibit some impulsive behaviors, such as compulsive shopping, binge eating, hypersexuality, and gambling. These behaviors may be related to a dose-dependent side effect of dopamine replacement treatment (Campagnolo et al., 2023; Chaudhuri et al., 2012; Poewe, 2008).

Several patients with Parkinson's disease are affected by olfactory dysfunction, which can be present months or even years ahead of the first motor symptoms. It seems that around 95% of patients exhibit a decreased sense of smell (hyposmia), and its severity is not dependent on the duration, treatment, or stage of Parkinson's (Antonini et al., 2023; Chaudhuri et al., 2012). Pain is another frequent non-motor symptom, which can sometimes cause more distress than motor symptoms. Usually, at the beginning of motor manifestations, PD patients complain of shoulder pain, which is typically related to limb rigidity, and it gets better when dopaminergic therapy starts. Pain in PD can also appear in patients who have motor fluctuations, off periods, and peak-dose dyskinesias (Antonini et al., 2023; Chaudhuri et al., 2012).

Sleep disorders are common in individuals with Parkinson's disease and can manifest even before the onset of motor symptoms, making them a potential risk factor for the disease. Insomnia and rapid eye movement (REM) sleep behavior disorder (RBD) are the most prevalent types. Patients with RBD may appear to be acting out their dreams due to a loss of REM sleep muscle atonia, which can result in violent body movements and jerking (Antonini et al., 2023; Chaudhuri et al., 2012; Poewe, 2008).

One of the most common gastro-intestinal symptoms in individuals with PD is constipation, which affects around two-thirds of patients. As it usually appears before the motor manifestation, constipation can also be considered a risk factor for Parkinson's Disease (Antonini et al., 2023; Chaudhuri et al., 2012).

It is important to assess non-motor symptoms of PD patients so we can monitor their progression, response to treatment, and consequences on the individual's quality of life. Some scales can be used for that, such as the Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part 1, which assesses the non-motor experiences of daily living, and the Scales for Outcomes in Parkinson's disease (SCOPA) set that can be used to assess various domains in PD: cognitive deficits (SCOPA-COG), autonomic dysfunction (SCOPA-AUT), sleep disorders (SCOPA-S), and psychiatric complications (SCOPA-PC) (Chaudhuri et al., 2012; Sauerbier et al., 2016).

Cognitive Impairment

Mild Cognitive Impairment and Dementia in Parkinson's Disease

Cognitive impairment is a common non-motor symptom of Parkinson's disease, being around six times more frequent in PD patients than in the healthy population (Aarsland et al., 2021). Because cognitive impairment can interfere in different domains (e.g., attention, visuospatial abilities, memory, and executive function), it affects the patients' function and quality of life, as well as their caregivers (Aarsland et al., 2021; Weintraub et al., 2022).

In Parkinson's disease, cognitive impairment is diverse in terms of the affected cognitive domain, the severity, and the rate at which it progresses, varying from subjective cognitive symptoms to mild cognitive impairment (MCI) to dementia (PDD). Some predictors of cognitive impairment include age of disease onset, presence of hallucinations, older age, depression, and severity of motor symptoms (Aarsland et al., 2021; Goldman et al., 2018; Zhang et al., 2020).

According to the International Parkinson and Movement Disorder Society, PD patients with mild cognitive impairment present a gradual decline in cognitive ability, but it shouldn't significantly impact the patients' functional independence and activities of daily living (Goldman et al., 2018; Zhang et al., 2020). This decline can be noticed by the patient, a caregiver, or the clinician, and can be assessed by a neuropsychological evaluation (Aarsland et al., 2021). The presence of MCI in PD could be a risk factor for dementia, as some patients progress to Parkinson's disease with dementia, but evidence shows that it may not always be the case (Goldman et al., 2018).

Dementia in Parkinson's disease is characterized as a cognitive impairment that affects two or more cognitive domains (visuospatial abilities, attention, executive function, and memory). This impairment must be severe enough to significantly impact the patient's normal functioning, affecting their activities of daily living and independence (Goldman et al., 2018). The risk of a PD patient developing dementia is high, around half of them develop PDD within 10 years, and around 80% at 20 or more years of the diagnosis (Aarsland et al., 2021; Biundo et al., 2016).

Neuropsychological assessment

Neuropsychological assessment is important to make a more accurate diagnosis of cognitive impairment in patients with Parkinson's disease. It can be used to assess memory, executive functions, attention, working memory, language, and visuospatial functions, or to make a global evaluation of the cognitive function (Aarsland et al., 2021).

The selection of which tests will be used during the neuropsychological assessment depends on some factors, such as the patient's age, language, education, and complaints. It is also important that the clinician takes into consideration that the performance in the tests can be affected by the motor and non-motor symptoms of Parkinson's disease, or even by the physiological changes that occur during aging (Goldman et al., 2018).

The Scales for Outcomes in Parkinson's Disease-Cognition (SCOPA-Cog) is a test that was created specifically to assess Parkinson's disease patients. It has appropriate construct validity and internal consistency, and it evaluates visuospatial functions, executive functions, memory, and attention. To assess longitudinal cognitive data or cross-sectional data in PD patients with mild cognitive impairment, the Cambridge Neuropsychological Test Automated Battery (CANTAB) is usually used (Brandão et al., 2020).

While a full neuropsychological assessment is time-demanding and expensive, a screening of cognitive function is faster, requires fewer resources, and may be less demanding. However, the screening is not as reliable as a neuropsychological assessment, and it also gives us less information (Aarsland et al., 2021; Brandão et al., 2020).

The Movement Disorder Society Rating Scales Review Committee recommended three scales for screening of cognitive functions, the Montreal Cognitive Assessment (MoCA), the Mattis Dementia Rating Scale Second Edition (MDRS-2), and the Parkinson's Disease Cognitive Rating Scale (PD-CRS). The most used of those scales in PD is the MoCA, as it has a specificity of 0.82 and a sensitivity of 0.41, with around 68% correct diagnoses of mild cognitive impairment in Parkinson's disease. A more detailed neuropsychological assessment can be done using the patients' scores on the MoCA (Aarsland et al., 2021).

The dual syndrome hypothesis

According to the dual syndrome hypothesis, the cognitive deficits in Parkinson's disease can be separated into two sets that are independent but partially overlap. In the first set (frontostriatal type), there is a dopamine-modulated dysfunction in the frontal-striatal network

that occurs in the early stage of PD, which causes problems in attention, planning, working memory, and response inhibition. In the second set (posterior cortical type), there is a posterior cortical dysfunction linked to cholinergic loss, which, if present, can lead to dementia. Usually, this last set leads to impaired visuospatial abilities, impaired recognition memory, and impaired language (Biundo et al., 2016; Brandão et al., 2020; Goldman et al., 2018).

Phases of Parkinson's Disease

It was suggested by the Movement Disorders Society (MDS) to divide Parkinson's Disease into three phases, the preclinical, the prodromal, and the clinical PD.

The preclinical phase is considered a pre-diagnostic phase. Here the pathology has already been initiated, there is the presence of neurodegenerative synucleinopathy, but no clinical symptoms are present. Currently, this phase can't be diagnosed, as there are no definitive biomarkers available for Parkinson's disease (Berg et al., 2014; Dommershuijsen et al., 2021; Noyce et al., 2016).

The prodromal phase is also considered a pre-diagnostic phase and the last one before the clinical diagnosis. In the prodromal phase, together with the neurodegenerative synucleinopathy, there is the emergence of some early symptoms and signs, mainly non-motor ones (e.g., REM sleep behavior disorder, constipation, and hyposmia), yet the diagnosis of Parkinson's disease is not possible as they are not specific to PD. At the moment, the diagnostic criteria for prodromal PD is probabilistic, as there isn't a way to identify it in a reliable way. It can be separated into probable prodromal PD, where there is a high likelihood (e.g., > 80%), and possible prodromal PD, where there is a lower likelihood of the neurodegenerative synucleinopathy (Berg et al., 2014; Dommershuijsen et al., 2021; Noyce et al., 2016).

Lastly, there is the clinical phase. Here the disease is fully established, and a diagnosis of Parkinson's disease is possible. The diagnosis is made based on the presence of the classical motor symptoms (bradykinesia, tremor, rigidity, and postural instability), with more details given in the next part (Dommershuijsen et al., 2021; Noyce et al., 2016).

Current Diagnostic Procedures

Today, the diagnosis of Parkinson's disease is made based on clinical criteria. The diagnosis is only confirmed postmortem, once there is identification of neuropathological changes in the brain, such as the accumulation of α-synuclein in Lewy bodies and Lewy neurites. At the beginning of the disease, it might be difficult to make a diagnosis of PD due to symptoms and signs overlapping with other forms of parkinsonism. Therefore, it is important that the patient's history is reviewed, and symptoms are assessed, to reduce the chances of diagnostic error and to rule out other disorders. (Bloem et al., 2021; DeMaagd & Philip, 2015; Jankovic, 2008). The International Parkinson and Movement Disorder Society's diagnostic criteria for Parkinson's Disease (MDS-PD) was created to be used in research, but it may also be used to help clinicians establish a clinical PD diagnosis (Bloem et al., 2021). According to the MDS-PD, first, we need to have a diagnosis of parkinsonism, therefore the patient must present with bradykinesia plus rest tremor or rigidity, or both. Once parkinsonism is identified, to determine whether PD is the cause of parkinsonism or not, the clinician must use three categories of diagnostic features:

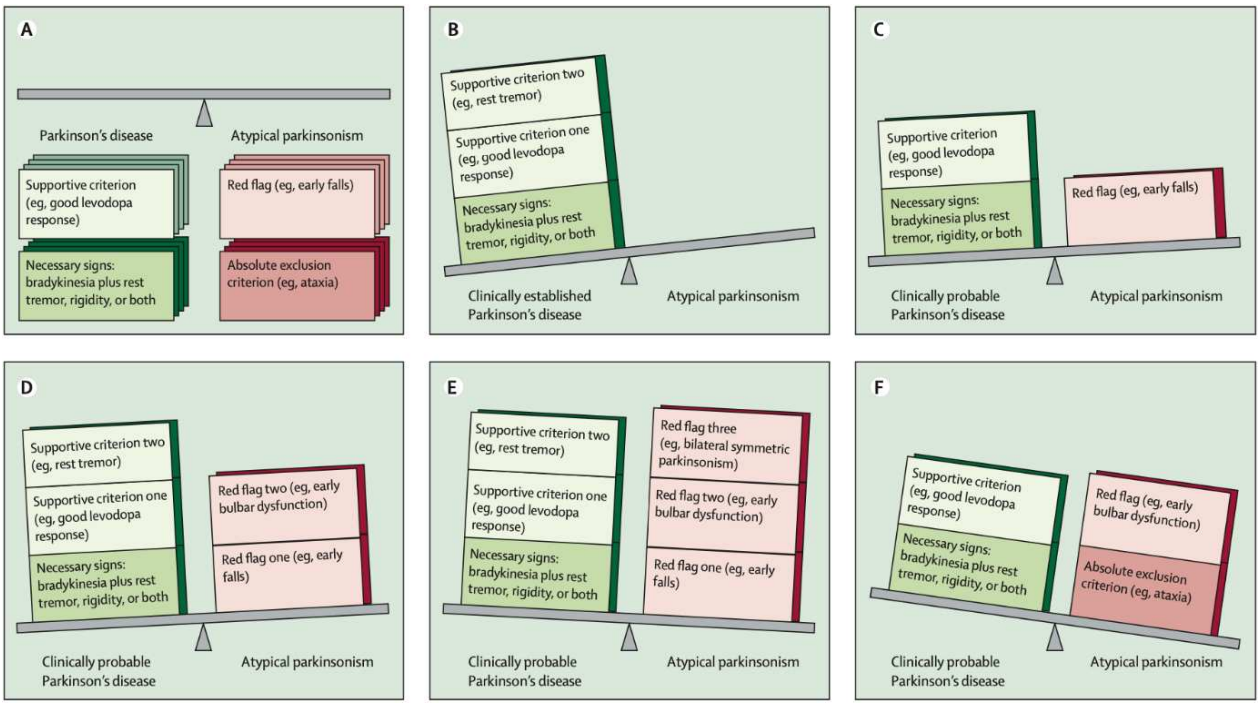
1. Absolute exclusion criteria: this helps rule out Parkinson's Disease as the cause of parkinsonism. According to Postuma et al., 2015, some examples of absolute exclusion criteria are "parkinsonian features restricted to the lower limbs for more than three years; absence of observable response to high-dose levodopa despite at least moderate severity of disease; and documentation of an alternative condition known to produce parkinsonism and plausibly connected to the patient's symptoms".
2. Red flags: to diagnose PD, if there are red flags, they must be counterbalanced by the supportive criteria. According to Postuma et al., 2015, some examples of red flags are a "rapid progression of gait impairment requiring regular use of a wheelchair within five years of onset; bilateral symmetric parkinsonism at onset; and recurrent falls because of impaired balance within three years of onset".

3. Supportive criteria: it is related to the positive features that improve the confidence of a diagnosis of PD. According to Postuma et al., 2015, some examples of supportive criteria are a “clear and dramatic beneficial response to dopaminergic therapy; rest tremor of a limb documented on clinical examination; and the presence of levodopa-induced dyskinesia”.

According to the MDS-PD, there are two levels of certainty of diagnosis for PD: clinically established Parkinson’s Disease, which maximizes specificity and reduces sensitivity, and clinically probable Parkinson’s Disease, which balances specificity and sensitivity (Postuma et al., 2015).

As can be seen in figure 4, to make a diagnosis of clinically established PD, it is required an absence of absolute exclusion criteria, at least two supportive criteria, and no red flags can be present. For a diagnosis of clinically probable PD, it is required the absence of absolute exclusion criteria. If there are red flags, they must be counterbalanced by supportive criteria (e.g., if one red flag is present, there must be one supportive criterion). Still, it is not allowed for more than two red flags to be present in this category (Postuma et al., 2015).

Figure 4. *Diagnostic criteria for Parkinson’s disease***.**



Note. The image shows the weighting process of the diagnostic criteria for clinically established and clinical probable Parkinson's disease according to the International Parkinson and Movement Disorder Society. From "Parkinson's disease", by Bloem, B. R., Okun, M. S., & Klein, C., 2021, *The Lancet, 397* (10291), 2284-2302, Figure 2 (https://doi.org/10.1016/s0140-6736(21)00218-x). CC BY.

Some neuroimaging techniques can be used to help diagnose PD, although they don't confirm the diagnosis. Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) help to differentiate Parkinson's disease from other disorders, such as essential tremor or drug-induced parkinsonism, by quantifying the reduction of Substantia nigra pars compacta dopaminergic nerve terminals projecting to the striatum (Gasser & Wichmann, 2023; Kalia & Lang, 2015).

Treatment today

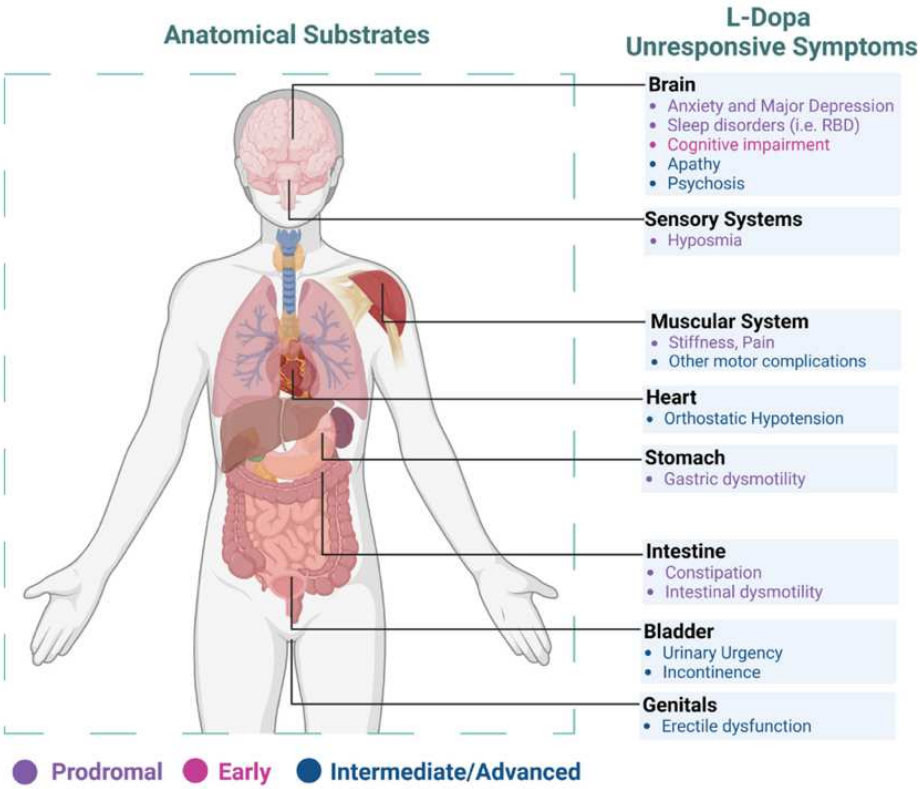
Symptomatic treatment

The treatment of Parkinson's disease today is aimed at managing the motor and non-motor symptoms of the disorder because there is no therapeutic intervention able to modify the progression of PD. The main treatment used is still dopamine replacement therapy with Levodopa (L-dopa) being the most prescribed drug, as it is the most efficient and tolerable antiparkinsonian drug available. Currently, one of the goals of research on Parkinson's disease is the development of a medication that will be able to either slow down or stop the neurodegenerative process of the disease. Some approaches target α-synuclein aggregations directly or indirectly (Kalia & Lang, 2015; Stocchi et al., 2024).

Levodopa, which use was initiated in the 1960s, is efficient in managing the symptoms of Parkinson's disease that are affected by the loss of dopaminergic neurons because of its mechanism of action. Once it reaches the brain, L-dopa is transformed into dopamine (DA) through decarboxylation (Dietrichs et al., 2023; Gasser & Wichmann). Some symptoms of PD

might not respond to Levodopa (figure 5) as they are not related to dopamine depletion. Usually, those symptoms are associated with the accumulation of α-synuclein misfolding in various tissues including the dopamine pathways, or because of dysfunctions that affect other neurotransmitters (Antonini et al., 2023).

Figure 5. *Levodopa unresponsive symptoms.*



From "Beyond the Dopaminergic System: Lessons Learned from levodopa Resistant Symptoms in Parkinson's Disease", by Antonini, A, Emmi, A, & Campagnolo, M, 2023, *Movement Disorders Clinical Practice, 10*(S2), Figure 1 (https://doi.org/10.1002/mdc3.13786). CC BY.

Treatment of PD patients usually begins when symptoms cause disabilities or discomfort, aiming to enhance function and quality of life in those individuals. As previously mentioned, Levodopa is the main drug used to treat motor symptoms of Parkinson's, but it is not the only one. Other medications may be used (table 1), like dopamine receptor agonists, monoamine oxidase type B inhibitors (MAO-B inhibitors), inhibitors of catecholamine ortho-

methyltransferase (COMT), and anticholinergic drugs (Gasser & Wichmann, 2023; Kalia & Lang, 2015).

Table 1. *Pharmacological treatments for motor symptoms and complications*

	Treatment of motor symptoms		Treatment of motor complications	
	Monotherapy	Adjunct to levodopa	Fluctuations	Dyskinesia*
Levodopa				
Levodopa-carbidopa	+	..	+	–
Levodopa-benserazide	+	..	+	–
Dopamine agonists (non-ergot)				
Apomorphine	–	+	+	–
Piribedil	+	+	–	–
Pramipexole	+	+	+	–
Ropinirole	+	+	+	–
Rotigotine	+	+	+	–
Dopamine agonists (ergot)				
Bromocriptine	+	+	+	–
Cabergoline	+	+	+	–
Monoamine oxidase type B inhibitors				
Rasagiline	+	+	+	–
Selegiline	+	–§	–§	–
Catechol-O-methyltransferase inhibitors				
Entacapone	..	+	+	–
Tolcapone	..	+	+	–
Others				
Amantadine	+	+	–	+
Anticholinergics†	+‡	+‡	–	–
Clozapine	+‡	+‡	–	+

+ indicates efficacious or likely efficacious. – indicates non-efficacious or insufficient evidence. .. indicates not applicable. *Responses to peak dose dyskinesia (diphasic dyskinesia might respond to drugs used for motor fluctuations, particularly dopamine agonists). †Includes benztropine, ethopropazine, trihexyphenidyl, and others. ‡For treatment of tremor. §There is insufficient evidence but, in practice, selegiline is used and can be effective.

From “Parkinson’s disease”, by Kalia, L. V., & Lang, A. E., 2015, *The Lancet, 286*(9996), 896-912, Table 2 (https://doi.org/10.1016/s0140-6736(14)61393-3). CC BY.

Dopamine receptor agonists can and have been used for some decades as monotherapies or together with levodopa. The most used types are pramipexole and ropinirole (oral drugs), rotigotine (transdermal drug), and apomorphine (injectable), and they all act on D2-like receptors. DA receptor agonists are effective in managing the cardinal features of Parkinson’s

disease, but they achieve fewer benefits when compared to levodopa. They also present more side effects, like psychosis, nausea, daytime sleepiness, orthostatic hypotension, and impulse control disorders, which are dose-dependent (Gasser & Wichmann, 2023; Kalia & Lang, 2015; Stocchi et al., 2024).

Rasagiline is a monoamine oxidase type B inhibitor used as monotherapy to reduce motor fluctuations in PD patients. It works by diminishing the breakdown of dopamine in the brain. MAO-B inhibitors are not as effective as levodopa or even as DA receptor agonists. One of the earliest antiparkinsonian drugs are the anticholinergic drugs, which works by blocking all subtypes of muscarinic receptors non-selectively. Because their effect on PD patients is poor when compared to levodopa and it also presents various side effects, anticholinergic drugs are not used much (Gasser & Wichmann, 2023).

Some catecholamine ortho-methyltransferase inhibitors used are entacapone, tolcapone, and opicapone. They work by blocking enzymatic degradation of levodopa in the periphery, therefore, increasing the effects of levodopa. When COMT inhibitors are used with levodopa, the wearing-off symptoms are reduced, and on-time is enlarged. COMT inhibitors may also present the same side effects as DA receptor agonists, with the addition of gastrointestinal disturbances (Gasser & Wichmann, 2023).

To determine when to start and what type of symptomatic treatment, the clinician should discuss it with the patient, to understand how it would impact the patient's lifestyle, their preferences, and their degree of disability (Stocchi et al., 2024).

Advanced treatment, motor fluctuations, and surgical treatment

Levodopa is the first-line therapy for PD patients, but after a few years of use, patients might develop medication-related fluctuations, wearing off (end-of-dose deterioration), and dyskinesias. This happens because as PD progresses, there is variance in dopamine levels in the brain related to intermittent Levodopa administration, diverse gastrointestinal function

and absorption, and escalating loss of dopamine nerve terminals and storage capacity (Antonini et al., 2023; Dietrichs et al., 2023).

Some strategies to deal with those issues can be to modify the dose and frequency of DA medication, increasing the doses of L-dopa, and diminishing the interval between them. If a combination therapy has not started yet, it can be initiated, with the addition of a MAO-B or a CAMT inhibitor. A patient who presents with dyskinesia can benefit from the implantation of amantadine (Antonini et al., 2023; Dietrichs et al., 2023).

Nowadays there are some advanced therapies available for when PD drugs cannot control the motor fluctuations and they become disabling, such as continuous infusion of dopaminergic drugs through a pump or the implantation of electrodes for deep brain stimulation (DBS) (Dietrichs et al., 2023).

The benefit of pump-delivery therapy is that it allows the medication to be administered at a constant rate, thus avoiding motor fluctuations, but its use is limited because of its acceptance by patients, cost, and tolerability. There are two ways in which it can be administered, infused subcutaneously with the DA agonist apomorphine, or through a percutaneous endoscopic jejunostomy tube. In this last one, levodopa is constantly delivered to the small intestine, either in the form of an intestinal gel that contains levodopa and the decarboxylase inhibitor carbidopa, or an intestinal gel that contains levodopa, carbidopa, and entacapone (COMT inhibitor) (Dietrichs et al., 2023; Stocchi et al., 2024).

The most invasive therapy is DBS. The patient must go through a neurosurgical operation to implement electrodes in the brain, usually in the subthalamic nucleus or in the midbrain. Today, the DBS system works in an open-loop way, in which the electrical stimulation can be delivered either continuously or in pre-programmed intervals (Dietrichs et al., 2023; Stocchi et al, 2024).

Deep brain stimulation is a well-established treatment for the cardinal motor features of Parkinson’s disease, but there are some contraindications such as atypical PD, dementia, and psychiatric disorders (Gasser & Wichmann, 2023; Kalia & Lang, 2015).

PART 2

Biomarkers: definition and importance

Today one of the biggest topics in neurodegenerative diseases research is the identification of biomarkers (Koníčková et al., 2022). According to the National Institutes of Health Biomarkers Definitions Working Group, a biomarker can be defined as "a measurable indicator of some biological state or condition that is objectively measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Li & Le, 2019).

Biomarkers can be used to assist in clinical diagnosis. They should help when estimating the current stage of the disease and its progression rate (Koníčková et al., 2022). In Parkinson's disease, currently, there are no biomarkers available for early detection of PD or that are sensitive to the progression of the disease (Li & Le, 2019).

There are some differences in terms of sensitivity and specificity of biomarkers. Specificity is related to the biomarker's capacity to precisely select cases in which the examined features are not present, while sensitivity is related to how successful a test is in detecting the conditions present that are perceived by the individual (Koníčková et al., 2022).

It is of great importance to detect an accurate and strong biomarker for Parkinson's disease, as it would assist in recognizing PD early, before the presence of motor symptoms; in making a differential diagnosis, such as between Parkinson's disease and other types of parkinsonism, or other neurodegenerative disorders; and in understanding therapeutic interventions and the efficacy of a treatment (Li & Le, 2019; Stocchi et al., 2024; Youssef et al., 2023).

Some potential biomarkers are being studied for Parkinson's disease diagnosis and progression, such as genetic (e.g., SNCA, PINK 1, GBA), biofluid and peripheral tissue (e.g., saliva, blood, CSF, skin biopsy), neuroimaging (e.g., PET, SPECT, MRI), among others (Sauerbier et al., 2016; Stocchi et al., 2024). There must be an appropriate and standard

procedure in those studies, to guarantee the reproducibility and quality of the results (Li & Le, 2019).

The ideal and most beneficial biomarker(s) would be reproducible, inexpensive, sensitive, noninvasive, and validated (Miller & O'Callaghan, 2015). The validation of a biomarker or a combination of biomarkers leads to the possibility of a screening campaign, to try to identify those individuals who may develop Parkinson's disease (Stocchi et al., 2024).

Classification of biomarkers

Biomarkers can be classified according to their applications. They can be separated into diagnostic, prognostic, monitoring, predictive, safety, susceptibility/ risk, and pharmacodynamic or response biomarkers (Parnetti et al., 2019).

- A diagnostic biomarker can identify or confirm the existence of a particular disease or condition, or recognize individuals with a subtype of the disease. This type of biomarker could be used to confirm a Parkinson's disease diagnosis or to differentiate PD from atypical parkinsonism, for example (Parnetti et al., 2019; Ryman & Poston, 2020).
- A prognostic biomarker can evaluate the possibility of a clinical event, disease recurrence, or advancement in individuals with the medical condition or disease of interest (Parnetti et al., 2019; Ryman & Poston, 2020).
- A monitoring biomarker can assess the status of a disease or medical condition by being measured repeatedly, or for confirmation of exposure to (or effect of) a medical product or an environmental agent (Parnetti et al., 2019; Ryman & Poston, 2020).
- A predictive biomarker can be applied to recognize individuals who are more likely than individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a medical product or an environmental agent (Parnetti et al., 2019).
- A safety biomarker is used to indicate the likelihood, presence, or extent of toxicity as an adverse effect. It is measured before or after exposure to a medical product or an environmental agent (Parnetti et al., 2019).

- A susceptibility/ risk biomarker stipulates the possibility of an individual, who at that moment, shows no clinically apparent disease or medical condition, to develop one in the future. This type of biomarker could be used to represent the potential of an individual developing Parkinson's disease, for example (Li & Le, 2019; Parnetti et al., 2019; Ryman & Poston, 2020).
- A pharmacodynamic or response biomarker can be used to show that a biological response, potentially beneficial or harmful, has occurred in an individual who has been exposed to a medical product or an environmental agent (Parnetti et al., 2019).

Parkinson's disease biomarkers

CSF and Blood biomarkers

α-synuclein

Alpha-synuclein (α-syn) is a synaptic protein present in large quantities in the thalamus, cerebellum, hippocampus, substantia nigra, and neocortex (Stocchi et al., 2024). Its main function is to regulate neurotransmitter release, synaptic function, and synaptic plasticity. In Parkinson's disease patients, α-synuclein can be seen abnormally in the patient's biofluids (e.g., saliva, blood, and cerebrospinal fluid), peripheral tissues, and organs (e.g., skin and gastrointestinal tract) (Chen et al., 2022).

α-synuclein has been studied in Parkinson's disease due to many reasons. First, α-synuclein is encoded by the SNCA gene, and the mutation of this gene has been associated with severe forms of PD. Secondly, the Lewy bodies and Lewy neurites seen in PD patients, are made of misfolded and aggregated α-synuclein. Lastly, as previously mentioned, α-synuclein has been found in CSF, blood, saliva, and gastrointestinal tract of living and deceased PD patients (Chen et al., 2022; Miller & O'Callaghan, 2015).

The primary cause of dopaminergic degradation in Parkinson's disease might be related to the misfolding and aggregation of α-synuclein. According to studies, α-synuclein aggregates have a prion-like spread, which means that the misfolded protein will induce misfolding in a

normal form of that same protein. Different factors such as gene mutation, post-translational modifications (PTMs), and clearance mechanism dysfunction, could lead to the misfolding and aggregation of α-synuclein into pathological forms (Chen et al., 2022).

- Gene mutation: a mutation on the SNCA gene can alter α-synuclein amino acid sequence, causing an alteration in its secondary structure from an α-helix to a β-sheet. This change can affect the aggregation rate and morphology (Chen et al., 2022).
- PTMs: some post-translational modifications that can affect aggregation and play a role in the misfolding of α-synuclein are ubiquitination, phosphorylation, and oxidization (Li & Le; 2019).
- Clearance mechanism dysfunction: in normal conditions, the body uses a clearance mechanism to remove the abnormal accumulation of α-synuclein. Lysosomes, proteasomes, glial cells, and others, play a role in this mechanism. In Parkinson's disease, this mechanism seems to be defective (Chen et al., 2022).

In the cerebrospinal fluid (CSF), α-synuclein can be separated into total α-synuclein, oligomeric α-synuclein, and phosphorylated α-synuclein. According to Parnetti et al., 2019, patients with PD have a lower CSF total α-synuclein when compared with healthy controls, but this might vary according to the patient's age, use of medication, disease stage, and so on. Therefore, CSF total α-synuclein is not a reliable diagnostic marker for Parkinson's disease, but it could offer promising results when used in combination with other CSF biomarkers. Parnetti et al., 2019 also stated that CSF oligomeric α-synuclein in Parkinson's patients is frequently seen at elevated concentrations when compared with controls, but the accuracy for diagnosis is not satisfactory.

Parkinson's disease is not the only disorder that presents with α-synuclein pathology, but at the moment, the misfolding, aggregation, and propagation of α-synuclein are considered one of the key factors driving the disease pathology (Stocchi et al., 2024).

MicroRNAs

MicroRNAs (miRNA) are small non-coding RNA molecules that are important in regulating gene expression at the post-transcriptional level (Esteves et al., 2022). MicroRNAs are seen as a promising biomarker in blood for detecting neurodegenerative diseases. In Parkinson's disease, microRNAs are seen as a possible biomarker for early detection and monitoring of disease progression (Li & Le, 2019; Koníčková et al., 2022).

According to Li & Le, 2019, some studies have shown that the modification of specific microRNAs could be relevant to the onset or progression of the disease. There also seems to be an involvement of microRNAs in the regulation of PD-related genes.

The most abundantly expressed miRNA in the brain is miRNA-124. It plays a role in neurogenesis, synapse morphology, neurotransmission, inflammation, autophagy, and mitochondrial function. A decrease in levels of miRNA-124 in plasma might be used as a potential diagnostic biomarker for PD (Koníčková et al., 2022).

When compared with controls, Parkinson's disease patients have different expression levels of miRNAs (Li & Le; 2019). In a paper published in 2022, Wei Chen's group compared 75 PD patients and 73 healthy controls. They investigated three microRNAs associated with α-synuclein expression in blood and noticed that a reduction expression of one of these microRNAs was correlated with a reduction score on the Unified Parkinson's Disease Rating Scale part 3, being able to differentiate PD patients and controls (Yamashita et al., 2023).

According to Koníčková et al., 2022, several studies noticed a downregulation of microRNAs at the first stages of neurodegeneration. This could mean that a decrease of miRNAs might not only just lead to dopamine-induced cell death, but also could contribute to the beginning of the biological process of neurodegeneration in Parkinson's disease.

MicroRNAs have been shown to be pertinent to the onset and progression of Parkinson's disease, but for miRNAs to be considered a biomarker used in clinical practice, more research is needed (Li & Le; 2019).

Alzheimer's disease biomarkers

Beta-amyloid 42 (Aβ42), tau, and phosphorylated-tau (p-tau) are Alzheimer's disease (AD) biomarkers. When assessing PD patient's cognitive decline and motor functions, those AD biomarkers can provide some insight into PD's progression. In some studies, it was seen that they were either normal or a bit reduced in the cerebrospinal fluid of Parkinson's patients (Yamashita et al., 2023).

According to Parnetti et al., 2019, although a reduction in CSF Aβ42 in PD patients is shown in some studies, this is not seen in other investigations. There is a trend in which CSF Aβ42 is elevated in patients with Parkinson's disease dementia (PDD) when compared to Alzheimer's disease patients. When compared to patients that have dementia with Lewy bodies, PD and PDD patients also present with an elevated CSF Aβ42 (Parnetti et al., 2019).

Neurofilament light chain

Neurofilament light chain (NfL) is a subunit of a neurofilament group. They are specific cytoskeletal neuronal proteins, predominantly found in unmyelinated axons. When there is axonal damage, it leads to the release of NfL into the CSF and blood (Koníčková et al., 2022). According to Parnetti et al., 2019, there is no strong evidence that Parkinson's disease patients show a change in the concentration of NfL in CSF and blood when compared with controls. However, CSF NfL concentration is elevated in patients who have atypical parkinsonian disorder when compared with PD patients (Parnetti et al., 2019; Yamashita et al., 2023).

Both blood and CSF neurofilament light chain have similar diagnostic accuracy, therefore, it might be a tool to assist clinicians in the differential diagnosis, helping to differentiate Parkinson's disease from atypical parkinsonian disorders (Parnetti et al., 2019).

Imaging biomarkers

DAT-SPECT

Dopamine transporter (DAT) single-photon emission computed tomography (SPECT) is an imagining technique used to detect the functioning of presynaptic dopamine transporters. It

can also be used to visualize the dopaminergic degeneration in the nigro-striatum of Parkinson's disease patients (Lotankar et al., 2017).

In 2011, it was approved by the Food and Drug Administration the use of DAT-SPECT scanning (DaTscan) Ioflupane I 123 injection as a tool for detecting Parkinson's disease (Yamashita et al., 2023). DaTscan, a radiopharmaceutical agent, is administered intravenously through a procedure named SPECT imaging. It may serve as a marker for dopamine terminal innervation because it binds to DAT proteins in the presynaptic membrane on dopaminergic terminals from the substantia nigra to the striatum (Lotankar et al., 2017). Research has shown that DAT proteins play an important role in the reuptake of dopamine from the synaptic cleft (Yamashita et al., 2023).

DAT-SPECT imaging could be a useful biomarker in unclear cases, helping to confirm or reject a diagnosis of Parkinson's disease (Yamashita et al., 2023). According to Kagi et al., 2009, if a patient with parkinsonism has an abnormal DAT-SPECT imaging, it supports the presence of nigrostriatal degeneration (e.g., Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration). However, if the patient has a normal DAT-SPECT, it supports an alternative diagnosis (e.g., drug-induced parkinsonism, vascular parkinsonism, essential tremor, or dopamine-responsive dystonia).

Currently, there is some evidence for the use of DAT-SPECT as a diagnostic biomarker for Parkinson's disease, but it is not clear the value of it as a prognostic biomarker. This could be related to some confounding variables, such as the progression of the disease, the patient's age, and antiparkinsonian medications (Yamashita et al., 2023).

F-DOPA PET

Similarly to DAT-SPECT, ^{18}F-DOPA L-6-[^{18}F] fluoro-3,4-dihydroxyphenylalanine (^{18}F-DOPA PET) is a helpful tool for the diagnosis of Parkinson's disease. ^{18}F-DOPA is an agent that measures the uptake of dopamine precursors and thereby assesses the integrity of presynaptic dopaminergic function. PET is a technique that quantifies, in the body's tissue, the metabolic activity of cells (Ibrahim et al., 2015; Yamashita et al., 2023).

^{18}F-DOPA PET is considered one of the best tools for a Parkinson's disease diagnosis in vivo, as it directly measures the synthesis of dopamine in the nigrostriatal pathway. Almost all patients with PD show, even at the first stages of the disease, a reduced ^{18}F-DOPA uptake in the putamen (Depierreux et al., 2021).

Even though ^{18}F-DOPA PET might be a helpful diagnostic biomarker for Parkinson's disease with positive results, there is still a need for research with big sample sizes. Additionally, access to ^{18}F-DOPA PET is restricted and exposes patients to radiation (Depierreux et al., 2021; Yamashita et al., 2023).

MRI

Magnetic resonance imaging (MRI) is a non-invasive imaging technique. Studies have indicated that MRI might be a good method to differentiate between Parkinson's disease and atypical parkinsonism (Aludin & Schmill, 2021; Yamashita et al., 2023).

Parkinson's disease patients, according to research, gradually show atrophy in different regions of the brain, such as basal ganglia, amygdala, hippocampus, parietal and occipital lobes, parahippocampal gyrus, and temporal gyrus. This atrophy can be seen with a structural MRI, using techniques such as T1 and/ or T2 weighted images, inversion recovery (IR), and magnetization transfer (MT). But, in PD patients with mild disease, it is not clear if there is a rise in brain volume, atrophy, or no change. Before considering structural MRI as a biomarker for Parkinson's disease, it is still needed studies with bigger sample sizes for it to be more accurately evaluated (Yamashita et al., 2023).

Neuromelanin MRI (NM-MRI) is an advanced MRI technique that has been studied as a potential biomarker for PD. Neuromelanin is a pigment protein that results from dopamine metabolism. It can be seen in certain regions of the brain, such as the substantia nigra, locus coeruleus, and nucleus of the solitary tract. NM-MRI can detect the content of neuromelanin, as well as indicate a reduction in the neurons that contain neuromelanin. Parkinson's disease patients have shown a reduced signal intensity of the substantia nigra on NM-MRI (Li & Le; 2019; Yamashita et al., 2023).

Currently, there is still little knowledge regarding the use of MRI to identify brain changes related to Parkinson's disease risk or prodromal PD (Yamashita et al., 2023).

Inflammation biomarkers

There is growing evidence pointing out that inflammation plays a role in Parkinson's disease pathology. In PD patients, neuroinflammation is related to the uncommon activation of microglia and altered levels of inflammatory mediators in the brain (Li & Le; 2019). Some potential inflammation biomarkers are IL-1β, IL-2, IL-6, IL-10, and TNF-α/ sTNFRs. They will be briefly described.

- IL-1β is a proinflammatory cytokine with pleiotropic biological actions in the peripheral blood and brain. According to Liu et al., 2022, a large study has shown that PD patients, when compared with controls, present with higher serum IL-1β levels, however, other studies did not find alterations in IL-1β levels in the serum and CSF of PD patients.
- Inflammatory processes in Parkinson's disease might be influenced by the gut microbiome composition, which can change cytokine profiles. IL-2 is able to suppress chronic inflammation in the gastrointestinal tract, as it plays an important role in Treg cell expansion, T cell proliferation, and mediation of inflammation-induced cell death. PD patients present elevated serum levels of IL-2, which can be reduced with antiparkinsonian medication (Liu et al., 2022).
- IL-6 is a cytokine with many functions and is mostly produced by neurons and glial cells. One of its functions is in neuronal development and differentiation, but IL-6 can also promote neuronal survival after damage or lead to neuronal death in neurodegenerative diseases. PD patients have higher levels of IL-6 in the striatum, CSF, and serum (Liu et al., 2022).
- IL-10 is an anti-inflammatory cytokine that lymphocytes and microglia produce. In PD patients, the levels of IL-10 in the serum are increased when compared to controls (Liu et al., 2022).

- Lastly, TNF-α is a proinflammatory cytokine that has an important role in host defense. It is able to activate microglia, leading to a progressive loss of dopaminergic neurons in the substantia nigra. PD patients have higher levels of TNF-α in CSF and serum (Liu et al., 2022).

Genetic biomarkers

SNCA

The first gene identified in Parkinson's disease was the SNCA gene, which has been linked with the familial form of PD. SNCA is the gene that encodes α-synuclein, therefore a mutation of this gene can increase the likelihood of oligomers or fibrils formation (Delamarre & Meissner, 2017; Mandel et al., 2010).

Parkinson's disease patients with SNCA mutations present with disease onset at an early age, quicker progression of motor symptoms, and notable non-motor symptoms. PD patients with a mutation in the SNCA gene respond to levodopa treatment, just like individuals with idiopathic PD (Bloem et al., 2021; Mandel et al., 2010).

LRRK2

Leucine-rich repeat kinase 2 (LRRK2) is a protein that has a role in vesicular trafficking, synaptic morphogenesis, and autophagy (Kalia & Lang, 2015; Yamashita et al., 2023). In Parkinson's disease, the LRRK2 gene is responsible for around 2 to 7% of sporadic PD in Caucasians, while in Ashkenazi Jews and North Africans, it is responsible for 40% of the cases (Stocchi et al., 2024).

The most frequent mutation in the LRRK2 gene is G2019S, which leads to an increase in kinase activity. Some studies have shown that this mutation can diminish the risk of cognitive impairment in patients with Parkinson's disease (Sauerbier et al., 2016; Stocchi et al., 2024).

GBA

GBA encodes for beta-glucocerebrosidase, which is a lysosomal enzyme. A mutation of the GBA gene can lead to Gaucher's disease and, currently, it is also known that it can be a risk factor for sporadic Parkinson's disease. PD patients who have a mutation of this gene can present with earlier disease onset as well as a severe course (Bloem et al., 2021; Sauerbier et al., 2016; Yamashita et al., 2021).

Parkin

Parkin is an E3 ubiquitin ligase that plays a role in the degradation of malfunctioning mitochondria. When there is a mutation, a loss of function can be seen (Delamarre & Meissner, 2017). In early-onset autosomal recessive Parkinson's disease, the most common mutation is in the Parkin gene. It can be seen in 15% of sporadic PD and about 50% of familial cases (Delamarre & Meissner, 2017; Kalia & Lang, 2015). PD patients who have a Parkin mutation usually present with early and more symmetrical onset with a slow progression of the disease (Sauerbier et al., 2016).

PINK1

PINK1 acts in a similar pathway to Parkin, being also involved in maintaining mitochondrial integrity and function in the dopaminergic neurons and muscles (Mandel et al., 2010). Mutations in the PINK1 gene are another frequent cause of autosomal recessive Parkinson's disease that leads to an early onset of PD (Bloem et al., 2021). The course of PD in patients who have this mutation is usually slowly progressive, but they tend to have a good response to antiparkinsonian treatment (Bloem et al., 2021).

DJ-1

Daisuke-Junko-1 (DJ-1) is a protein from the PARK7 gene. DJ-1 not only assists in protecting the brain during oxidative stress, but it also can inhibit the aggregation of α-synuclein monomers. A mutation of the PARK7 gene can induce a significant decrease in DJ-1, which is

seen in Parkinson's disease patients, leading to an increase of α-synuclein aggregates (Yamashita et al., 2023). Clinically, a mutation in DJ-1 is similar to PINK1 and Parkin mutations, causing an early age of disease onset (Delamarre & Meissner, 2017).

Microbiome

Recent advances in clinical and preclinical research on Parkinson's disease have indicated the important role of the gut-brain axis in the disease (Emmi et al., 2023). The gut-brain axis refers to a bidirectional communication system. On one side, the microbiome in the gut can interfere with the brain's activity and functionality by sending neuronal signals to the brain by the vagus nerve, as well as by stimulating the enteric nervous system. On the other side, the brain can influence gut functions through stress response (e.g., anxiety), affecting gut motility, secretions, and immune response (Elfil et al., 2020).

Due to the participation of the enteric nervous system in the early stages of Parkinson's disease and its connection to gut motility, it is important to detect α-synuclein aggregation and deposition in gut tissues. According to animal model studies, there might be a bidirectional transmission of α-synuclein pathology which can either begin in the enteric nervous system and disseminate to the brain, or the other way around, starting at the brain and going towards the periphery (Emmi et al., 2023).

Enteric glial cells (EGCs) seem to have an important part in the exchange between inflammation and neurodegeneration. Research suggests they are crucial in the pathophysiology of gastrointestinal disorders, as they partake in the regulation of gastrointestinal functions. In Parkinson's disease patients, elevated levels of expression for enteric glial markers in the gastrointestinal tract have been observed, which could suggest a response of enteric glial cells (Emmi et al., 2023).

Studies have also shown that Parkinson's disease patients may experience a condition called "leaky gut syndrome", which seems to be related to enteric α-synuclein pathology (Caputi & Giron, 2018; Tan et al., 2022). In this condition, the gut barrier function is damaged, allowing

an elevated number of microbes and molecules, such as bacteria and toxins, to cross the intestinal epithelium, which can trigger inflammation and oxidative stress. The resulting inflammatory state might increase α-synuclein expression, misfolding, and aggregation, potentially leading to a local pro-inflammatory immune response, generating a feedback loop that facilitates the spread of α-synuclein (Caputi & Giron, 2018; Tan et al., 2022).

Best biomarker for Parkinson's disease

Currently, there isn't a single definitive biomarker for Parkinson's disease as none of the biomarkers explained above were able to show an accurate and early diagnosis. Therefore, the diagnosis of PD is still made clinically and is only confirmed post-mortem (Li & Le; 2019; Yamashita et al., 2023). However, research has shown that combining multiple biomarkers, such as imaging, CSF, and blood, with clinical assessment can lead to an improvement in the diagnostic accuracy of Parkinson's disease (Li & Le, 2019; Parnetti et al., 2019).

It is of extreme importance to find a definitive biomarker for Parkinson's disease, as it would help in diagnosis reducing the subjectivity and facilitating the differential diagnosis (e.g., atypical parkinsonism), as well as helping in treatment development (Yamashita et al., 2023).

Conclusion

In conclusion, there still isn't a definitive biomarker that can detect Parkinson's disease early or that is sensitive to the progression of the disease. However, the identification of a biomarker for PD is one of the major topics in neurodegenerative research at the moment (Koníčková et al., 2022). Some of the potential biomarkers that are being studied for Parkinson's disease include cerebrospinal fluid and blood biomarkers, such as α-synuclein, microRNAs, neurofilament light chains, tau, beta-amyloid 42, and phosphorylated-tau; neuroimaging biomarkers, such as MRI, DAT-SPECT, and ^{18}F-DOPA PET; microbiome; inflammation biomarkers, such as IL-1β, IL-2, IL-6, IL-10, and TNF-α/ sTNFRs; and genetic biomarkers, such as SNCA, DJ-1, PINK1, LRRK2, GBA, and Parkin (Sauerbier et al., 2016; Stocchi et al.,

2024). While none of these biomarkers can yet be considered a definitive biomarker for Parkinson's disease, studies have indicated that a combination of multiple of those biomarkers (e.g., imagining, genetic, CSF and blood biomarkers) can increase diagnostic accuracy, enhance early detection of the disease, and facilitate in the differentiation of PD from atypical parkinsonism or other neurodegenerative diseases (Li & Le, 2019; Parnetti et al., 2019).

Parkinson's disease, currently, is the second most common neurodegenerative disease and the fastest-growing neurological disorder globally. The diagnosis of PD is still primarily made based on clinical observation, and the confirmation of the diagnosis is only possible post-mortem (Li & Le, 2019; Sobel, 2015). Therefore, it is of great importance the discovery of a biomarker that is accurate and strong for Parkinson's disease, as it can help clinicians to recognize PD early (before the appearance of the motor symptoms when there already is 40 to 60% of striatal dopamine nerve terminals lost), as well as in making a differential diagnosis (e.g., differentiating Parkinson's disease from atypical parkinsonism). An accurate biomarker for PD could guide the development of new treatments for patients, and help monitor the treatment efficacy (Antonini et al., 2023; Li & Le, 2019; Youssef et al., 2023). Overall, the identification of a biomarker for PD could help improve the quality of life of the patients, and potentially help in slowing disease progression (Sauerbier et al., 2016; Li & Le, 2019).

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