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Final Degree Project

PROBIOTICS AS AN ADD-ON TREATMENT FOR PARKINSONS' DISEASE PATIENTS SUFFERING FROM MOTOR FLUCTUATIONS AND DYSBIOSIS

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"It is important to realize that if certain areas of science appear to be quite mature, others are in the process of development, and yet others remain to be born."

- Santiago Ramón y Cajal, Advice for a Young Investigator

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1. ABSTRACT:

PROBIOTICS AS AN ADD-ON TREATMENT FOR PARKINSONS' DISEASE PATIENTS SUFFERING FROM MOTOR FLUCTUATIONS AND DYSBIOSIS

Background: Parkinson's disease is the second most common neurodegenerative disease in our setting. It is characterised by the onset of motor symptoms, but patients present with a wide range of non-motor symptoms. Gastrointestinal symptoms are very common, constipation and delayed gastric emptying are common complaints of patients because of an early impairment of the enteric nervous system. In addition, many studies have demonstrated the presence of gut dysbiosis in PD patients, although it is not yet known whether it is a cause or a consequence. Regardless, PD patients often develop a condition known as small intestine bacterial overgrowth. This has been shown to be one of the causes of motor fluctuations in levodopa-treated PD patients; and its treatment improved them. However, because predisposing factors were still present, relapse rates were substantially high. Recently, there has been increased interest in the benefits of using probiotics in PD patients to treat these gastrointestinal complaints. By restoring the gut microbiota, patients regain normal gut function. In preclinical and clinical studies, probiotics have been shown to improve both motor and non-motor scales and overall quality of life in PD.

Objective: the main objective of this clinical trial is to demonstrate that the use of probiotics as a post-treatment of antibiotics (rifaximin) in PD patients suffering from small intestine bacterial overgrowth and motor fluctuations reduces the relapse rate and provides an improvement in levodopa efficacy, compared to patients receiving placebo alone after standard treatment.

Design: this will be a multicentre, randomized, double-blind, placebo-controlled, parallel-group study in all Catalan hospitals with a motor disorders department.

Intervention and Methods: subjects of the study will be patients with Parkinson's disease suffering from motor fluctuations and small intestine bacterial overgrowth. Our sample size will be of 432 patients. All of them will be treated with antibiotics, and after they will be randomized in two groups: intervention group (n=216), in which patients will be taking probiotics; and placebo group (n=216). the intervention group (taking probiotics) and 216 in the control group (taking placebo).

Keywords: Parkinson's disease, non-motor symptoms, dysbiosis, small intestine bacterial overgrowth, probiotics

2. ABBREVIATIONS:

AADC	Aromatic L-amino acid decarboxylase
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
CEIC	"Comité de Ética e Investigación Clínica"
CFU	Colony-forming units
CNS	Central nervous system
COMPT	Catechol-o-methyltransferase
DA	Dopaminergic agonists
DBS	Deep brain stimulation
eGP	External globus pallidus
ENS	Enteric nervous system
GBT	Glucose breath test
GDNF	Glia-derivate neurotrophic factor
GI	Gastrointestinal
H&Y	Hoehn and Yahr
HB	*Helicobacter pylori*
Hs-CRP	High-sensitivity C-reactive protein
IBS	Irritable bowel syndrome
LB	Lewy bodies
LBT	Lactulose breath test
LDR	Long duration response
LN	Lewy neurites
LPS	Lipopolysaccharides
MA	Multisystemic atrophy
MAO-B	Monoaminoxidase B
MI	Main investigator
MPTP	1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine
NG	Neurologist
NMS	Non-motor symptoms
PD	Parkinson's disease

PM	Project manager
PIGD	Postural instability and gait disturbance
PSP	Progressive supranuclear palsy
RBD	Rapid eye movement sleep behaviour disorder
REM	Rapid eye movement
ROS	Reactive oxygen species
ST	Statistician
SCFA	Short chain fatty acids
SDR	Short duration response
SIBO	Small intestine bacterial overgrowth
SNpc	Substantia nigra pars compacta
STN	Subthalamic nuclei
TDC	Tyrosine decarboxylase
UDysRS	Unified Dyskinesia Rating Scale
UK-PDSBB	UK Parkinson's Disease Society Brain Bank Diagnostic Criteria
UPDRS	Unified Parkinson's Disease Rating Scale
WHO	World's health organization

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4. BACKGROUND:

4.1. Parkinson's disease:

4.1.1. Definition:

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the **substantia nigra pars compacta** (SNpc), resulting in an impaired dopaminergic system function in the striatum. This loss leads to a multisystemic onset of symptoms within which the rigid-akinetic syndrome with resting tremor is highlighted (1).

4.1.2. Epidemiology:

PD is an age-related disease, with incidence and prevalence increasing gradually over the years. However, it does not exclusively affect older people since the onset for almost 25% of affected individuals is younger than 65 years old, and for 5-10%, younger than 50. In our setting, it is the second most common neurodegenerative disease after Alzheimer's, and its prevalence is estimated to be around 1% of the population above 60 years old. PD incidence ranges from 5/100,000 to over 35/100,000 new cases per year (2,3).

There are no outstanding epidemiological differences worldwide. Moreover, while it affects both sexes, the incidence in women is lower, and their age at onset is higher than in men. Women also have a higher risk of developing dyskinesia and motor and non-motor response fluctuations and are more likely to report depression. On the other hand, men live a higher number of years with disability and have a greater risk of cognitive deterioration (2).

4.1.3. Aetiology:

The etiopathology of PD is, at present, still unknown. However, there are four relevant factors when talking about this disorder.

- First of all, we can consider the **genetic factor**. There is a reasonable comprehension of the causative genes, and up to 10% of cases have shown a typical mendelian transmission pattern. As of yet, most research is focused on SNCA, LRRK2, PRKIN, PINK1, and GBA genes (1,4). Most PD cases are sporadic; however, the importance of the genetic influence in these cases is becoming more relevant. More than 20 *loci* are known to increase PD's risk (1). These are the most relevant ones:

  - **PARK-SNCA (PARK1):** This gene encodes the α-synuclein protein involved in vesicle trafficking, docking, priming and fusion, neurotransmitter release, and axonal transport. It is the main component of **Lewy bodies** (LB) and **Lewy neurites** (LN).
  - **PARK-PARKIN (PARK2):** It is the most common AR PD-related gene. The clinical features of this mutation are usually symmetrical. Usually, patients have a remarkable levodopa response, although levodopa-induced dyskinesia also tends to appear quite early. At autopsy, there is loss of neurons in SNpc, but the dorsal tier is preserved and LB are rarely present.
  - **PARK-LRRK2(PARK8):** It is the most common AD PD-related gene. Most of LRRK2 mutations are of late-onset, resembling typical PD. However, they have a more benign course, without **rapid eye movement sleep behaviour disorder** (RBD), and more of a **postural instability and gait disturbance** (PIGD) phenotype. Some atypical features are orthostatic hypotension, dementia, hallucinations, corticobasal syndrome, and primary progressive aphasia.
  - **PARK-GBA:** Glucocerebrosidase (1q21) encodes the lisosomal enzyme glucocerebrosidase, that decomposes glucocerebroside into glucose and ceramide, and has a role in sphingolipid degradation.

- Secondly, there is the **environmental factor**. The environment is in constant flux; thus, its assessment is unlikely. The environmentome is the sum of all potentially causative and protective factors present in our environment, and there are several elements we can take into account. Heavy metal exposure, pesticides, insecticides (dieldrin), rural living, **1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine** (MPTP), rotenone… They are not determinative, but it seems they increase susceptibility (4,5).
- Another critical element is **aging**. Aging seems to be the most significant independent risk factor in PD. Many of the mechanisms of the cascade of events leading to cell death take place parallel to the aging process (1,6).

- And the last, **the interactions thereof.** PD idiopathic variant is the most frequent one, and it seems to be a result of the interaction between natural factors such as aging, environment and, genetics (4).

4.1.4. Pathophysiology:

PD happens due to a neuronal loss in SNpc, locus coeruleus, ventral tegmental area, and other dopaminergic systems. However, this degeneration does not happen equally everywhere, and these susceptibility differences are related to the properties of each neuronal population. Remarkably, the dopaminergic neurons in SNpc possess a pace-like activity, increasing their vulnerability (1). Furthermore, loss of dopaminergic neurons relates mainly to motor symptoms. However, we also have the presence of extranigral pathology, defined as a spectrum of **non-motor symptoms** (NMS) (7).

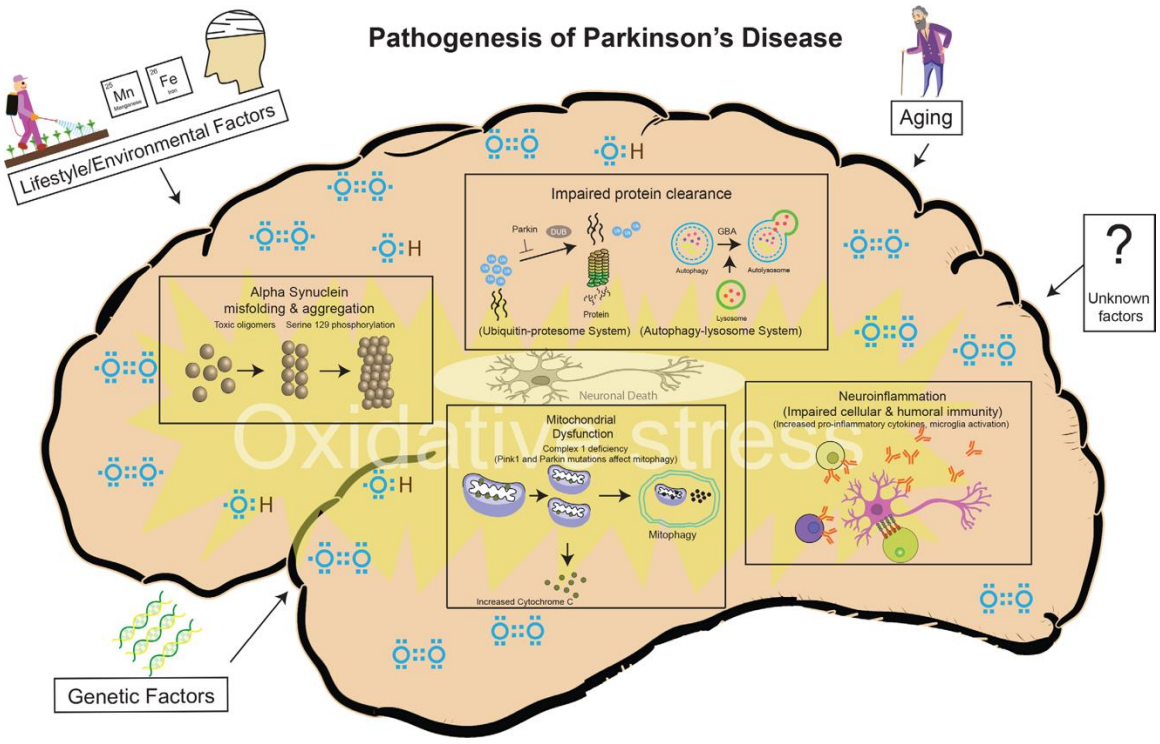

Figure 1.*Pathogenesis of PD (4).*

There are various **pathophysiologic mechanisms**, yet quite a few critical molecular phenomenona and hallmarks have been consistently reported (4).

- **Abnormal α-synuclein aggregation:** The α-synuclein protein has been found to be toxic to dopaminergic neurons. Its conformational changes and its aggregation are influenced by elements such as oxidative stress, PD gene mutations or overexpression of α-synuclein. There are various α-synuclein forms, some are able to activate neuroinflammatory responses or spread α-synuclein pathology from cell to cell (4). It has been proposed that α-synuclein can self-propagate in a prion-like manner, transferring the pathology to unaffected cells by promoting misfolding of the normal α-synuclein (8). A defined set of peptides derived from α-synuclein act as possible antigenic epitopes and drive helper and cytotoxic T-cell responses. Approximately 40% of people with PD have shown immune responses to α-synuclein (5).

- **Oxidative stress and mitochondrial dysfunction:** Oxidative stress occurs by an overproduction of **reactive oxygen species** (ROS), and this event leads to mitochondrial damage. As a result, there is a decrease in mitochondrial complex 1 activity, leading to the release of cytochrome C and the caspase cascade's activation, resulting in a cessation of mitochondrial metabolism and ultimately evolving to cell death. In addition, mitochondrial damage leads to the accumulation of oxidized dopamine and reduced glucocerebrosidase (1,4).

- **Impairment of protein clearance:** It entails the ubiquitin-proteasome and the **autophagy-lysosomal** systems. In normal conditions, abnormal, mutated, or damaged proteins are ubiquitinated and broken down into a proteasome. This system works with **adenosine triphosphate** (ATP), provided by mitochondrial metabolism. In cases of α-synuclein overproduction, the proteasome can get oversaturated. When the mitochondrial metabolism, because of genetic or environmental causes, does not work correctly, with an increase in oxidative stress and an ATP deficiency, the proteasome cannot break down appropriately the proteins. These oligomers are toxic to the neurons; they accumulate as LB and LN, leading to neural death (1).

- **Neuroinflammation:** It used to be seen as a response to neurodegeneration. However, studies have shown that there is an **impaired cellular and humoral immunity**, with an increase of pro-inflammatory cytokines, changed immune cell population and microglia activation. There is also evidence that diseases with peripheral inflammation present a higher PD risk (2,4).

- **Gut microbiota dysbiosis:**

  The **microbiota-gut-brain axis** communicates the **central nervous system** (CNS) and the **enteric nervous system** (ENS). It includes several elements: the CNS, the autonomic nervous system, the ENS, the neuroendocrine system, and the gut microbiota (9).

  The gut microbiota is the community of microorganisms in the human **gastrointestinal** (GI) tract that live in symbiotic interaction with their host. They contribute to essential functions such as extraction, synthesis, and absorption of nutrients and metabolites like bile acids, lipids, amino acids, vitamins, and **short-chain fatty acids (SCFAs)**. In addition, microbial products are involved in the development of the CNS (9,10). Intestinal SCFAs (formic acid, acetic acid, propionic acid and butyric acid) play a pivotal role in modulating the gut immune response and the preservation of the intestinal barrier function and **blood-brain barrier** (BBB). The decrease in SCFAs' may promote intestinal inflammation and increase the risk of α-synuclein deposition in the gastrointestinal tract and the ENS (9).

  The dominant phyla in gut microbiota are Firmicutes, composed of different genera such as *Lactobacillus, Bacillus, Clostridium, Enterococcus,* and *Ruminococcus*; and Bacteroidetes, predominantly consisting of *Prevotella*. Both account for 90% of the microbial population. We also have Actinobacteria phylum, less abundant, represented by the *Bifidobacterium*; Proteobacteria phylum with *Enterobacteriaceae* such as *Escherichia* and *Shigella*, and *Campylobacterales*; Fusobacteria phylum; and Verrucomicrobia phylum with *Akkermansiaceae* (10). Gut microbiota varies based on the intestine anatomical regions according to physiology, pH and oxygen levels, digesta flow rates, substrate availability, and host secretions. Furthermore, the gut microbiota is subjected to individual changes that depend on age, type of delivery, milk feeding methods, use of antibiotics, body mass index, ethnicity, dietary habits, exercise frequency, and the presence of certain disorders (10). A **gut microbial dysbiosis** is an imbalance in the guts' microbiota structure, function, or both, and it can promote pathological changes in the CNS in PD (9). For instance, recent studies have revealed a decrease in *Prevotellaceae, Lachnospraceae, Clostridiaceae* (*Clostridium cocoides*), and *Bacteriodaceae* (*Bacteroides fragilis*) families and an increase in *Verrucomicrobiaceae, Lactobacillaceae, Enterobacteriaceae* and *Enterococcaceae* families (9,11–13).

Figure 2. *Taxonomic gut microbiota composition(10).*

Prevotellaceae is involved in the production of intestinal mucus and SCFA. Therefore, a decreased level of mucin production might lead to increased intestinal permeability, growing the risk of intestinal inflammation. Additionally, *Prevotellaceae* decrease has also been correlated to a reduced **ghrelin** concentration (9,13,14). Besides its orexigenic effect, instigating gastric acid secretion, and increasing gastric motility, studies in mice have proven that ghrelin plays a significant role in preserving the nigrostriatal pathways.

Firstly, it improves the dopamine availability because of its effect on the firing rate of SNpc dopaminergic neurons. On the other side, it makes these neurons more resistant to cellular stress by changing the mitochondrial respiration and ROS production (15). Evidence has shown that plasma levels of ghrelin in PD patients are low (9).

Intestinal inflammation allows the leakage of **microbial products**, causing a peripheral inflammation that could trigger α-synuclein aggregation in the ENS. One of them are the **lipopolysaccharides** (LPS), on account of an augmentation of the gram-negative bacterial population. Evidence proved that this metabolite reduced the number of tight junction proteins of the zona ocludens 1 and e-cadherin intestinal epithelial cells in mice. On the other hand, faecal microbiota of PD patients shows a decrease in the production of SCFAs (9,16). Furthermore, high calprotectin levels in PD patients have also been found, which indicates an intestinal barrier dysfunction. The rise in pro-inflammatory cytokines (IL-1ß, IL-6, IL-17, TNF- α, IFN-γ) caused by systemic inflammation causes a BBB impairment, contributing to **neuroinflammation**. Moreover, these events trigger α-synuclein aggregation both in the CSN and the ENS (9).

According to Braak's hypothesis (8), early pathological changes do not occur in the CNS but in the medulla oblongata, the olfactory bulb and the ENS, and that would explain early symptoms such as hyposmia and GI alteration. Injection of human α-synuclein into the gut tissue of healthy rodents induced α-synuclein aggregation first within the ENS, and then the vagus nerve and brainstem. According to this hypothesis, the gut could be acting as a path for the spread of synucleinopathy. In fact, GI dysfunctions are usually the first symptoms appearing in PD patients, constipation being present in nearly 80% of them. With its increase in gastrointestinal transit time, gastrointestinal dysfunction has also been linked to **small intestinal bacterial overgrowth** (SIBO). This condition is associated with the deterioration of GI symptoms and motor functions, mainly because it disrupts drug absorption by causing modifications in the metabolism of levodopa (7,9). There is evidence that *Lactobacillus* (*Lactobacillus brevis*) and *Enterococcus* (mainly *E.faecium* and *E.faecalis*), overgrowing in PD, remarkably reduce the levels of levodopa/decarboxylase inhibitor in these patients because of their ability to decarboxylate levodopa to dopamine through **tyrosine decarboxylases** (TDC). And the usual decarboxylase inhibitor associated with levodopa in the general treatment is not able to inhibit bacterial TDC (17).

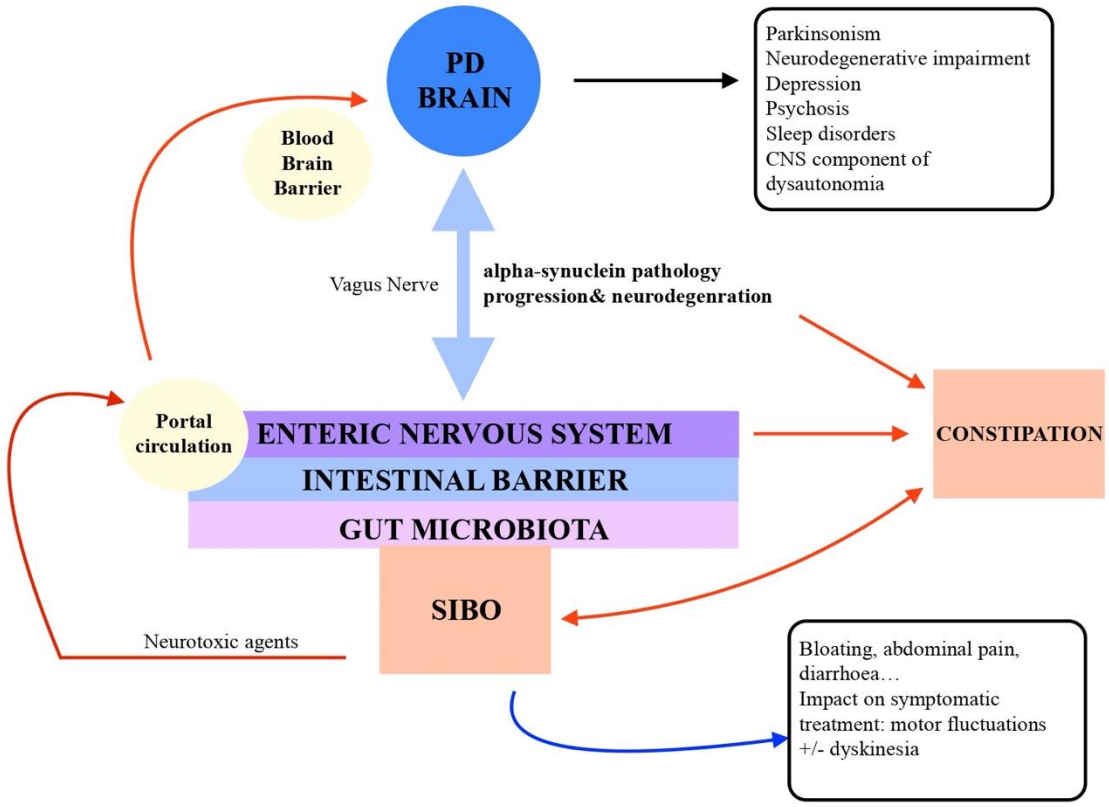

***Figure 3**. Relationship between SIBO and PD. Adapted from(18).*

- **Other major molecular and cellular hallmarks:**

  Vesicular transport disruption, loss of microtubular integrity, neuronal excitotoxicity, disruption of trophic factors, iron metabolic pathway dysregulation, endoplasmic reticulum impairment, poly (ADP-ribose) polymerase and other enzymatic activation.

All this comes together in one thing, the reduction of dopamine on the basal ganglia. The **death of the nigrostriatal dopaminergic cells** causes an imbalance between direct and indirect pathways, with an impairment in the neural connections between the basal ganglia, the thalamus, and the motor cortex. Consequently, bradykinesia and other parkinsonism manifestations appear (1). Dopamine is a dual modulator and contributes to two possible circuits on the basal ganglia. The direct pathway with D1 neurons inhibits the output nuclei to stimulate movement. On the other hand, the indirect pathway, with D2 neurons, inhibits the **external globus pallidus** (eGP) and the **subthalamic nuclei** (STN) to stimulate the output nuclei and stop the movement. In PD, there is a higher activity of the output nuclei, thus, a higher activity of the indirect pathway (19).

4.1.5. Clinical features:

Traditionally, PD starts when the cardinal motor symptoms appear. However, other structures not directly involved in motor control are also affected. Thus, patients manifest a broad spectrum of NMS that tend to occur many years before the motor symptoms start (1).

4.1.5.1. Non-motor symptoms:

- **Hyposmia:** It is defined as the loss of smell ability, usually present from the start of the illness, affecting around 90% of the patients. It is related to α-synuclein aggregation in the olfactory bulbs and the neuronal loss of the central olfactory pathways. Although it is not specific to PD, it allows the differentiation between vascular parkinsonism and PD (1,20).

- **Dysautonomy:**

  - **GI symptoms:**

    a) Swallowing disorders: PD patients suffer from oropharyngeal and oesophageal dysphagia related to the bradykinesia and rigidity associated with the disease. This dysfunction increases the risk of aspiration, increasing the risk of upper respiratory tract infection and pneumonia, one of the significant causes of death in PD patients (21).

    b) Constipation: Constipation is the most frequent complaint in PD patients, long before the motor symptoms start. Up to 80% of PD patients report it. Studies have shown abnormal aggregates of α-synuclein in the ENS and the dorsal motor vagus nerve. However, PD treatments such as anticholinergics and dopamine agonists are also responsible for it. This condition leads to delayed colonic transit and dyssynergic defecation.

c) Small intestinal bacterial overgrowth (SIBO): SIBO is a malabsorption syndrome associated with an increase of bacterial density above 10^5 **colony-forming units** (CFU)/mL of small intestinal aspirate. Clinical manifestations are bloating, flatulence, abdominal pain, nausea, dyspepsia, and diarrhoea. Its prevalence in PD patients is high, and studies have shown that patients suffering from SIBO have more severe motor fluctuations(21). Furthermore, the presence of SIBO affects drug metabolism and absorption. SIBO happens due to small bowel dysmotility resulting from LB pathology in the peripheral autonomic nervous system and ENS because of PD, causing constipation and the ensuing overgrowth. However, medications also contribute to it. In addition, it is believed that SIBO might also contribute to reduced gastric emptying through inflammatory effects on enterochromaffin-like cells (18). Management is based on the use of oral antibiotics to eradicate the small intestine's overpopulation, usually consisting of rifaximin from 7 to 14 days (18).

d) Motility disorders: A delayed gastric emptying leads to postprandial pain, bloating, vomiting, and weight loss. Furthermore, gastroparesis symptoms impact the delivery of PD medication because these drugs are absorbed in the small intestine. As noted above, there is evidence of α-synuclein aggregations in Meissner's and Auerbach's plexuses. Studies have also shown a decrease in ghrelin levels in PD patients (21).

e) Malnutrition: Dyskinesias, an important adverse effect of PD medication, can cause weight loss by increasing energy spending, and low body weight increases dyskinesias risk. **Levodopa** affects fat metabolism, skeletal muscle glucose uptake, hormones, homocysteine levels, and vitamin B6 and B12 levels.

- **Loss of respiratory sinus arrhythmia and orthostatic hypotension:** There is sympathetic heart denervation in most patients, leading to a dysfunction in the arterial baroreceptor reflex. At the beginning of the disease, they are usually not symptomatic, but the appropriate tests display them (1,20).

- **Urogenital dysfunction:** A detrusor overactivity increases the urinary frequency, with micturition urgency and bladder dysfunction in almost a 32% of the patients. This category also includes erectile and ejaculatory failure, widespread but not very intense. Most cases appear in the late stages of PD (1,20).

- **Sleep disorders:** It is one of the most frequent NMS in PD. In the beginning, sleep fragmentation and a reduction in non-REM sleep. However, PD neurodegeneration has an impact on sleep structure and induce REM sleep disorders. As a result, patients suffer from a loss of REM atonia, with tonic activity during their sleep that usually is related to dream content. Other sleep issues are nocturnal cramping, painful dystonia, restless legs syndrome, night-time incontinence, hallucinosis, and daytime sleepiness (1,20).

- **Neuropsychiatric dysfunction:**
  a) Depression: Some features are anxiety, panic attacks, loss of interest and initiative, fatigue, indecisiveness, and anhedonia. In some cases, they precede motor symptoms. It could happen as a reaction to the diagnosis, but the multiple transmitter deficiencies in the mesocortical and mesolimbic pathways play an essential role (20).
  b) Psychosis: They are frequently related to cognitive decline and dementia, the use of parkinsonian drugs, and sleep disorders. Visual hallucinations are common in the late stages, usually colourful and rich in detail(20).
  c) Impulse control disorders: Related to PD treatment, especially dopamine agonists. Levodopa dysregulation syndrome leads patients to a progressive abusive increase in levodopa. Furthermore, **deep brain stimulation** (DBS) leads to an impairment of the frontostriatal dopaminergic system. Both can lead to inappropriate behaviour (1).

- **Cognitive impairment and dementia:** They are highly identified in PD, affecting almost 80% of the patients after 15-20 years of diagnosis. It is usually related to frontal executive dysfunction because of multifactorial neuropathology. Dementia in PD patients is related to a more rapid progression of disability. There is impaired problem-solving, goal-directed behaviour, set shifting, visuospatial deficits, psychomotor slowing, apathy, bradyphrenia, fluctuations in attention and cognition, mood and personality disorders, hallucinosis and psychosis, and deficits in memory retrieval (1,20).

- **Pain:**

  It is very common and has different aetiologies (1,20).

  a) Musculoskeletal pain: It is related to rigidity and hypokinesia. Tendinitis or bursitis in the knees, hip, and shoulder are frequent, and it could be because of the asymmetry.

b) Dystonic pain: Dystonia in *OFF* periods (return of PD symptoms between medications' doses) or sudden dystonia can cause pain.

c) Neuropathic pain: It does not present with a neural distribution but a regional one. Described as a gnawing pain dysesthesic pain, it appears because of an impairment in central pain-processing pathways.

4.1.5.2. Motor symptoms:

- **Bradykinesia:** Bradykinesia is the slowness of voluntary and automatic movement, and one of the cardinal symptoms in PD. It happens due to the imbalance between the direct and indirect pathways in the basal ganglia, causing an imbalance in oscillatory rhythms. As a result, there is too much akinetic ß activity and poor prokinetic γ activity (1,5). In the beginning, it is highlighted in activities requiring fine movements or arm swinging movements when walking. Furthermore, as the patient repeats the movement, the range of it lowers (hypokinesia), and so does the rhythm. In advanced PD, it also manifests as hypomimia, ocular motor disorders, hypophonia, and micrography (1).

- **Rigidity:** Rigidity is another cardinal symptom of PD and is experienced as muscle stiffness. This condition impairs joint mobility throughout all of its range. In its early stages, it manifests in axial muscles. Later on, distal muscles become affected too (1).

- **Tremor:** Tremor is the third cardinal symptom in PD. It occurs mainly at rest and lessens during sleep. It usually begins asymmetrically, the hand being usually the first structure to be affected, followed by the leg on the same side. However, the tremor can also appear on other body parts such as the neck or the jaw (1).

- **Abnormal gait and posture disorders:** Parkinsonian gait is defined by small, shuffling, and low steps. However, sometimes patients seem to walk at accelerating speed (festinating), unable to stop. This condition leads to a higher risk of falls in patients suffering from PD. Other changes are increased steps when turning around, decreased arm swing when walking, or sudden freezing episodes of the gait.

> Posture disorders are more frequent in the late stages of PD, and patients adopt a stooped posture. It is related to the disease's gravity. The bend can be very prominent at the waist area; in those cases, we define it as camptocormia. If the affection is very asymmetric, patients tilt sideways as well (1).

Various scales have been created to stage the functional disability associated with PD according to its progression. On clinical praxis, the most used ones are the **Hoehn and Yahr Scale** (H&Y) (ANNEX I) and the **Unified Parkinson's Disease Rating Scale** (UPDRS). Both of them rate motor symptoms, however, NMS are also important to take into account since they affect the patient's quality of life as well. Scales allow a better assessment of the disease and, thus, better management (19).

4.1.6. Diagnosis:

PD diagnosis is mainly clinical, based on the patient's medical history and a proper neurological examination. The diagnostic criteria recommended for PD is the **UK Parkinson's Disease Society Brain Bank Diagnostic Criteria** (UK-PDSBB). In addition, it contains a section of exclusion criteria for PD in order to avoid diagnosis mistakes. As healthcare professionals, it is also essential to consider that many drugs, such as neuroleptics, antiarrhythmics or antidepressants, can also cause parkinsonism symptoms.

Besides the clinical symptoms and physical examination, some diagnostic tests are used to rule out other clinical entities when the patient does not manifest the cardinal parkinsonian symptoms or during the first stages of the disease. For example, **neuroimaging** techniques allow the exclusion of tumours, vascular diseases, or other disorders similar to PD, like **progressive supranuclear palsy** (PSP) or **multisystemic atrophy** (MA).

A more specific neuroimaging technic is the **Spect Brain DaTscan**, revealing the brain's dopamine levels in a more targeted manner, thus, allowing an accurate diagnosis. On the other hand, when the age at onset is early (younger than 50 years old), or there is a positive family history, **a genetic diagnosis** is also recommended (23).

Table 1. *UK Parkinson's disease Society Brain Bank Diagnostic Criteria (UK-PDSBB).* Modified from *(23)*

UK Parkinson's Disease Society Brain Bank Diagnostic Criteria (UK-PDSBB)
First step. Parkinsonism diagnosis

- Bradykinesia
- At least one of the following:
  o Muscular rigidity
  o Resting tremor of 4-6 Hz
  o Postural instability not caused by primary visual, vestibular, cerebellar, or proprioceptive impairment

Second step. Exclusion criteria for Parkinson's Disease
- Repeated strokes with stepwise progression - Repeated head injury - Definite encephalitis - Oculogyric crises - Use of neuroleptic or dopamine-depleting agents at onset of symptoms - One or more relatives affected - Sustained remission - Strictly unilateral features after 3 years - Supranuclear gaze palsy - Cerebellar signs - Early severe disautonomy - Early severe dementia with disturbances of memory, language or praxis - Babinski sign - Cerebral tumor or communicating hydrocephalus on neuroimaging - Negative response to large doses of levodopa (malabsorption excluded) - Exposure to known neurotoxin (e.g., MPTP)
Third step. Supportive criteria for PD (3 or more are required for definite diagnosis)
- Unilateral onset - Resting tremor - Progressive disorder - Asymmetrical onset of symptoms - Excellent response (70-100%) to levodopa - Severe levodopa-induced chorea - Sustained levodopa response for 5 years or more - Clinical course of 10 years or more - Hyposmia - Visual hallucinations

4.1.7. Treatment:

4.1.7.1. Parkinson's disease first stage treatment:

PD treatment is based on dopaminergic drugs. Various factors, particularly age, guide the choice of the drug used in PD patients. In addition, other items should also be considered such as the gravity of motor symptoms at diagnosis, the affective disorders, or the socio-familiar situation of the patient.

It is considered that patients above the age of 70 should start treatment with **levodopa**. On the other hand, in patients under that age, the use of **dopaminergic agonists** (DA) is prioritized. Furthermore, patients who suffer from a lower degree of motor impairment might also start with **monoaminoxidase B** (MAO-B) inhibitors or amantadine.

- **Levodopa:**

  Levodopa is the most effective and powerful antiparkinsonian drug available. It is a prodrug of dopamine (Levo-2,3-dyhydroxyphentanylananine). Its absorption happens in the proximal one-third of the small intestine, and later on, it is transported across the BBB by an active transporter. Its plasma half-life is of 60-90 minutes. (23). It is imperative to avoid its metabolism on peripheral tissues by decarboxylase enzymes. That is why it always needs the presence of **aromatic L-amino acid decarboxylase** inhibitors (AADC inhibitors), such as **benserazide** or **carbidopa**. Therefore, nowadays, all commercially available levodopa formulations contain AADC inhibitors (23).

  The bioavailability of oral levodopa depends on gastric emptying. However, this process is delayed in some PD patients, most notably those suffering from motor fluctuations. Furthermore, we must consider that this drug may also be involved in this phenomenon, as well as the constipation. Therefore, inconsistent gastric emptying modifies the levodopa concentration-time curve, triggering fluctuations in its pharmacokinetics (24).

  Anyhow, in the early stages, motor improvement is more significant than levodopa plasma level fluctuations. Despite that, each doses' efficacy and duration decrease proportionally to the disease's progress. This phenomenon happens because of various reasons that will later be discussed (24).

It is, however, worth mentioning, that levodopa's therapeutic response consists mainly of two components(25):

→**The short-duration response** (SDR): Single doses of levodopa improve motor disease for a couple of hours.

→**The long-duration response** (LDR): An extended administration of levodopa has shown a persistent antiparkinsonian effect that appears to be constant over time. This, however, does not happen, as we shall explain later.

- **Dopaminergic agonists:** Dopaminergic agonists (DA) are effective as monotherapy in early stages – especially in younger patients – and in combination with levodopa. Ergot derivatives were the first ones to be used, but since there is a higher risk of heart valve fibrosis, non-ergoline DA are nowadays on first line. Pramipexole and ropinirole are administered orally, rotigotine transdermally, and apomorphine subcutaneously (23).

- **Monoaminoxidase B inhibitors:** Monoaminoxidase B inhibitors (MAO-B inhibitors) reduce dopamine metabolism by inhibiting MAO-B, an enzyme found in platelets and the CNS (the glia). This allows an increase in dopamine levels in the CNS. Selegiline and rasagiline are irreversible inhibitors; safinamide is a reversible one (23).

- **Catecol-O-methyltransferase inhibitors:** Levodopa formulations contain AADC inhibitors to improve the bioavailability, albeit less than expected. That is because of levodopa's catabolism in the gut by **catechol-o-methyltransferase** (COMPT) enzymes. COMPT inhibitors are specific drugs – entacapone, tolcapone, and opicapone – that increase the levels of levodopa by avoiding its breakdown. They help treat motor fluctuations but have been shown to increase the frequency of dyskinesias. (23).

- **Amantadine:** Amantadine is an antiviral drug with multiple mechanisms of action. It has anti-glutamatergic effects by inhibiting NMDA receptors, anticholinergic effects, raises the levels of D2 in the striatum, and also increases the expression of **glia-derivate neurotrophic factor** (GDNF). The guidelines recommend its use to treat dyskinesias related to levodopa (23).

- **Safinamide:** Safinamide is an α-aminoamide with dopaminergic and nondopaminergic effects; it inhibits MAO-B enzymes, blockades sodium channels, and modulates the release of glutamate. It is recommended to treat and reduce motor fluctuations (23).

- **Apomorphine:** Apomorphine is a D1 and D2 agonist, and it is used in advanced PD as intermittent subcutaneous injections or continuous subcutaneous infusions. In these advanced cases, apomorphine is a rescue therapy for *OFF* periods and allows better control of motor fluctuations (23).

4.1.7.2. Motor complications. Fluctuations and dyskinesias:

As the disease progresses, the efficacy of PD treatment decreases and motor complications begin to appear. These consist primarily of **motor fluctuations** and **dyskinesias** (23).

Pathophysiology of the motor complications:

Motor fluctuations happen due to several reasons:

→Central pharmacokinetic factors: Levodopa does not prevent dopaminergic neuron degeneration; hence the striatum's ability to store presynaptic dopamine decreases. Because of that, a decay of the LDR happens, losing the striatum's capacity to buffer the oscillations in plasma levodopa levels. Therefore, PD patients begin to suffer from a high dependence on external levodopa and its SDR. It is also important to highlight the preserving activity of MAO and COMPT enzymes (25).

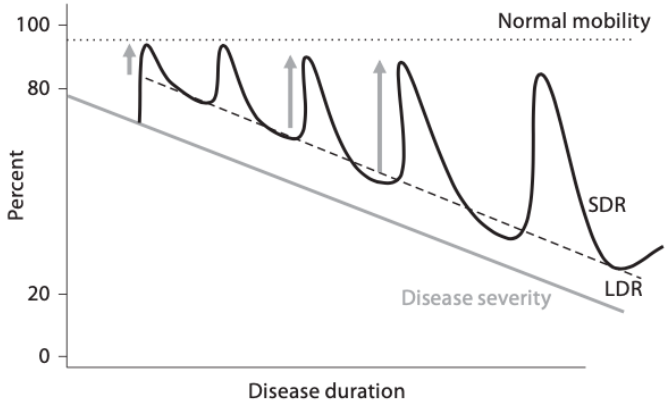

***Figure 4**. Schematic depicting the decline in the LDR and its inverse relationship to the magnitude of the SDR(25).*

→Peripheric pharmacokinetic factors: Peripheric factors modify levodopa plasma levels by disrupting its absorption. Thus, its bioavailability is impaired(23).

→Pharmacodynamic factors: Levodopa is a short-acting agent, and because of that, there is an abnormal stimulation of dopaminergic receptors. Instead of a continuous stimulation, there is a pulsatile one (23).

On the other hand, when talking about **dyskinesias**, it is important to consider these components as well (23):

- D1 receptors become hypersensitive because of their pulsatile stimulation.
- Serotoninergic pathways transform exogenous levodopa to dopamine in an uncontrolled manner.
- There is an abnormal response of striatum's GABA neurons, activating the direct pathway.
- There is a higher glutamatergic activity from the brain cortex to the striatum.

Motor fluctuations:

- **Wearing-*OFF*:** OFF periods are defined as the return of symptoms between levodopa doses. The wearing-*OFF* phenomenon is a standard and predictable response of any pharmacological system when activated by a short-acting agent. In PD's case, parkinsonian symptoms reappear at the end of each levodopa dose. It is mainly related to levodopa's plasmatic levels (23).
- **Sudden unpredictable *OFF*:** Parkinsonian symptoms appear unexpectedly and are unrelated to levodopa intake. It is more common in the late stages of the disease (23).
- **Failure of dose; partial or delayed response:** The levodopa dose does not reach the expected benefit. This happens either because of a delayed response (*delayed ON*); the response is sub-optimal (*partial ON*); or because it is absent (*dose failure* or *no ON*). What this does is that, as time goes on, patients end up needing more daily levodopa doses (23).
- **Morning akinesia:** Parkinsonian symptoms appear when waking up because the last dose was insufficient (23).

- **Beginning of dose worsening:** There is a transitory worsening of the symptoms the first minutes after levodopa intake, mainly the tremor (23).
- **End of dose rebound:** There is an aggravation of parkinsonian symptoms at baseline without treatment and at the end of dose. Highly infrequent (23).
- ***ON-OFF* fluctuations/Yo-Yoing:** Random oscillations between ON and OFF. This phenomenon can be predictable or unpredictable (23).

Dyskinesias:

Dyskinesias are defined as abnormalities in voluntary movement. They take place because of the dopaminergic agents' chronic treatment. Its clinical expression is variable, with chorea and dystonia being the most prevalent and ballism and myoclonus being a much rarer occurrence (23). They can be classified in two main groups (23):

- **High levodopa dose dyskinesias:**

    - Peak-dose dyskinesia: In the *ON* state, one to two hours after levodopa's dose. These are choreic movements that first happen around the neck and later spread around the torso and the limbs. For more severe cases, ballistic movements can also appear. These usually occur by the end of the day because of the cumulative levodopa dose. Therefore, by reducing the daily doses, this phenomenon can be improved. But this will be at the expense of increasing parkinsonian symptoms.
    - Plateau dyskinesia: It correlates with the plateau levodopa levels.
    - Ocular dyskinesia: They manifest as oculogyric crises along with the peak-dose dyskinesias.
    - Respiratory dyskinesia: Expressed as irregular breathing rhythm in peak-dose dyskinesias.
    - Myoclonus: Is uncommon, usually 10 to 20 minutes after levodopa dose. It usually happens in patients who suffer from dementia.

- **Low levodopa dose dyskinesias:**

  They consist of dystonia episodes happening during the *OFF* or wearing-*OFF* period, mainly around the lower limbs, which is painful for the patient. Furthermore, it causes deformities that hinder the patient's gait.

Treatment:

Modifying levodopa's frequency and the dose is a standard practice to treat motor complications. For instance, clinical practitioners can shorten the interval between levodopa doses when patients suffer from wearing-*OFF* episodes. On the other hand, the amount of levodopa should be reduced to improve dyskinesia occurrence (23).

Small and frequent doses reduce levodopa's plasma variability. However, as the disease progresses, the threshold of levodopa concentration to reach therapeutic effects increases. Furthermore, frequent doses can worsen evening dyskinesias due to sustained levodopa levels(23).

As stated above, wearing-*OFF* episodes are mainly related to levodopa's bioavailability. Therefore, drug combinations are significant assets to keep stable levodopa plasma levels. Many patients require add-on treatments, such as MAO-B and COMT inhibitors, to improve motor fluctuations without worsening dyskinesia episodes (23). Safinamide, an MAO-B inhibitor, can be particularly highlighted for its dopaminergic and nondopaminergic mechanisms. Evidence has shown that the addition of safinamide 50mg/day or 100 mg/day allows a significant improvement in *OFF* time, with no or non-troublesome dyskinesia, as well as an improvement in the UPDRS motor scale (26).

4.1.8. Prognosis:

Even if, over the last years, PD symptomatic treatment has improved significantly, the disease's progress worsens the prognosis. Dopaminergic drugs have managed to increase patients' livelihoods from 10 to 20 years and bring the mortality rate closer to the general population (although it is still 1.5 times higher).
Over time, the patients' functional status and quality of life deteriorate because of a decreased sensitivity to levodopa and because other symptoms, resistant to standard treatments, appear, such as falls, sleep disorders, and dementia.

4.2. Probiotics:

As we have previously mentioned, there is evidence from human and animal studies demonstrating the presence of gut dysbiosis in PD. A **dysbiosis** is defined as a situation in which, in a specific microbial ecosystem, the predominant microorganisms are not the ones usually found in it. Consequently, the beneficial impact of healthy gut microbiota is reduced (27). It is, however, unclear whether it is a cause or a consequence of PD pathogenesis. Still, as a result, there is a neuronal affection in both the ENS and the CNS caused by the following inflammatory cascades and oxidative reactions (28).

Probiotics are defined by the **World Health Organization** (WHO) as *"living microorganisms that, when administered in adequate amounts, confer a health benefit on the host"*(28).

Probiotics are beneficial thanks to their ability to restore gut microbiota and, therefore, maintain immune homeostasis by enhancing intestinal epithelial integrity (mainly through SCFA); protecting the host against gut barrier disruption; regulating the immune system and GI mucosa through their immunomodulatory effect; inhibiting pathogenic bacterial growth through their microbiological barrier; and even by modulating the brain function (28).

A healthy gut microbiota can maintain these functions thanks to specific metabolites they synthesize (9,29):

- Short chain fatty acids (SCFA): They include formic, acetic, propionic, and butyric acid. They play vital roles in our organism. Studies have shown their ability to reduce proinflammatory cytokines such as TNF-alpha and IL-17 and increase anti-inflammatory cytokines like IL-10. Thus, allowing an improvement in intestinal inflammation. SCFA also increase the expression of tight junction claudins and downregulate TLR4, improving the function of the intestinal barrier. On the other hand, there is evidence of their capacity to modulate neuroinflammation and reduce dopaminergic neurons degeneration. By upregulating **brain-derived neurotrophic factor** (BDNF) and **glial cell-line derived neurotrophic factor** (GDNF), they prevent the activation of astrocytes and microglia and regulate neuroinflammation (29,30).

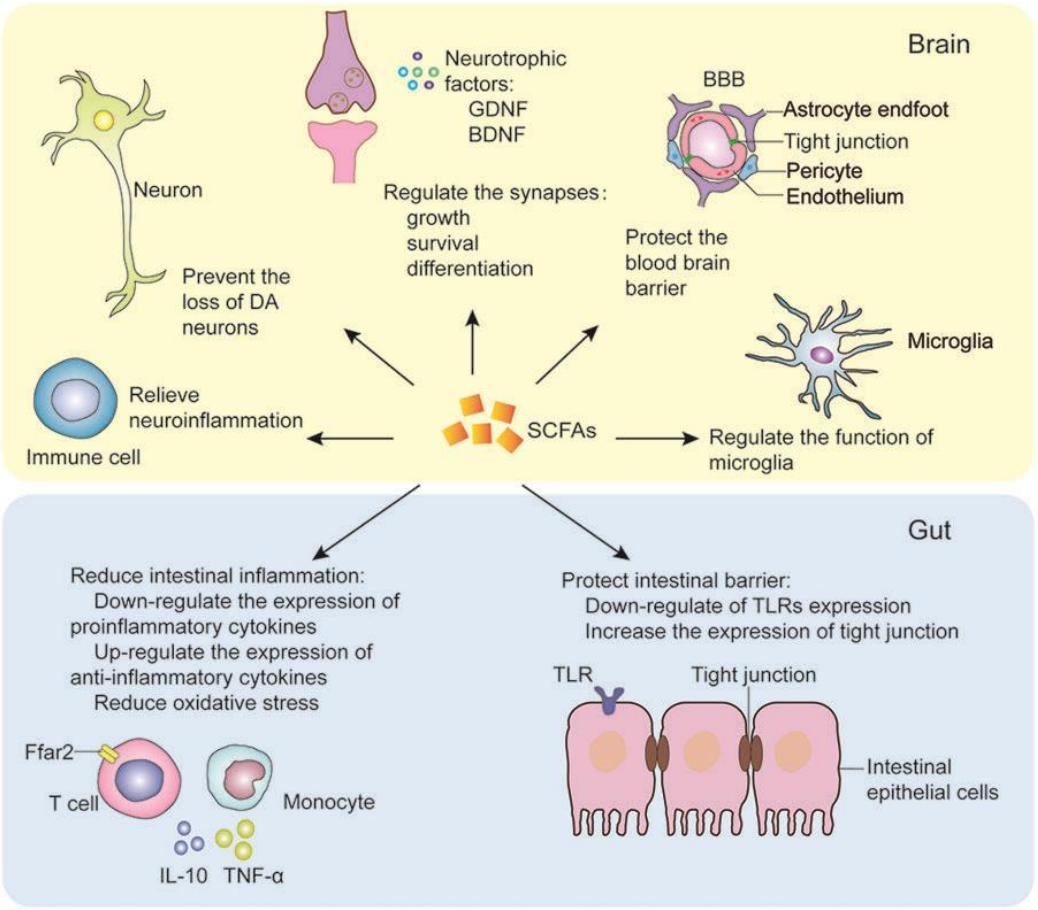

Figure 5. *Involvement of SCFA in the gut-brain axis in Parkinson's disease (9).*

- Bile acids: Bile acids are produced through cholesterol in the liver, and microbiota metabolizes them as well. In the intestinal tract, they eliminate bacterial overgrowth and diminish bacterial translocation and endotoxemia, protecting the small intestine from their adverse effects while preserving the epithelial barrier. Bile acids also play a neuroprotective role by eliminating the toxic effect of ROS and inhibiting the activation of astrocytes and microglia (9).

- Neurotransmitters: About 90% of our organism's serotonin levels are found in the GI tract, either absorbed from the diet or produced by the gut microbiota. The kynurenine pathway is the primary route of tryptophan catabolism, through which serotonin levels are regulated. This neurotransmitter has a significant role in epithelial secretion, intestinal peristalsis, vasodilation, and maintenance of enteric neurons (9,29).

- Ghrelin: Ghrelin is produced in the GI tract. Research has shown a high amount of ghrelin receptors in the hippocampus, the SN, the raphe nuclei, and the ventral tegmental area. Furthermore, ghrelin has demonstrated an ability to reduce ROS accumulation and protect mitochondrial integrity. On the other hand, ghrelin receptors on SN neurons stimulate tyrosine hydroxylase expression, heightening dopamine production (29).

For all that, the CNS can also affect the gut microbiota by several signalling molecules involved in regional motility, secretion of acid, production of bicarbonates and mucus, maintenance of epithelial fluid, the permeability of the intestine, and the mucosal immune response. Most of them are thanks to the sympathetic and parasympathetic influences on the circuits of the ENS.

In Spain, there is a consensus on probiotics accessible in pharmacy, their use, and the degree of recommendation for several dysbiosis conditions. To bring a successful probiotic to market, it takes several steps to choose the proper stain and ensure it is safe. To date, most genera are *Lactobacillus* and *Bifidobacterium* since no genes related to pathogenicity have been described yet; in opposition to *Enterococcus*, which can lodge several virulence factors (such as hemolysin, gelatinase…). Because of that, *Enterococcus* strains have not been included in the **Qualified Presumption of Safety**[1] list. Besides, *Enterococcus* strains have shown the ability to increase levels of some biogenic amines like histamine or thiamine. These molecules could induce GI, circulatory or respiratory disorders, especially in patients with lower levels of MAO. And if we take into consideration PD patients, *Enterococcus* strains have recently been found to be able to inactivate levodopa by their TDC enzyme activity(17). As examples, the guidelines recommend the use of *L.acidophilus, L.casei subsp.paracasei, L.delbrueckii subsp. Bulgaricus, L.plantarum, B.infantis, Bifidobacterium breve,* and *S.thermophilus* mixtures to treat functional constipation or **irritable bowel syndrome**(IBS). Or in children suffering from regurgitation, *L.reuteri* probiotics evidenced the ability to accelerate gastric emptying (27,31)

[1] A safety assessment procedure for microbes used in the food chain.

So far, probiotics have been used to manage PD in several pre-clinical and clinical studies. Intake of probiotics containing *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus reuteri,* and *Lactobacillus fermentum* for 12 weeks has proved to improve some symptoms in PD patients, with beneficial impacts on MDS-UPDRS, but also by decreasing the **high-sensitivity C-reactive protein** (hs-CRP), insulin levels, and oxidative stress through the increased production of SCFA in the gut (32).

Other studies have focused on improving constipation in PD patients; one in particular (33) used a multi-strain probiotic containing *Lactobacillus sp* and *Bifidobacterium sp* at 30 $x10^9$ CFU. Patients evidenced that spontaneous bowel movements increased following this intervention, and so did constipation.

5. JUSTIFICATION:

PD is a neurodegenerative disorder characterized by the loss of dopaminergic neurons in the nigrostriatal pathway, causing the motor symptoms that patients typically present. However, other structures not involved in motor control are also affected, manifesting a broad spectrum of NMS. Among them, GI symptoms are of great relevance, not solely because of their implications on the patient's quality of life, but because they usually occur long before the motor symptoms do (1,4).

Many studies have shown that α -synuclein aggregation, the main histopathological finding in PD, occurs in the ENS much earlier than in the CNS. There is nothing confirmed yet regarding the cause, but there is enough evidence to suspect on a gut dysbiosis as the main origin, a very frequent phenomenon in PD patients (7,9,11,12,28). It is believed that this dysbiosis would be responsible for the intestinal barrier disruption, causing an inflammation leading to the formation of α -synuclein aggregates in the enteric plexus; α -synuclein has been shown to act in a prion-like manner and would be able to travel to the CNS through the vagus nerve (4,5). In addition, cytokine release from systemic inflammation would affect the BBB function, spreading inflammation at the CNS level and contributing to neuronal loss (9). Whether it is a cause or a consequence is still unclear. Still, GI abnormalities have been found in PD patients, leading to two clinical conditions that are highly frequent in them: constipation and delayed gastric emptying. And both favour the occurrence of SIBO, a type of gut dysbiosis significantly prevalent in PD patients due to intestinal dysmotility and possibly altered host immune function.

To date, PD's treatment is symptomatic, being levodopa the drug that has shown greater benefits. However, in the long run, PD patients start to suffer from motor fluctuations. These are treated by raising levodopa's dose and frequency, at the price of important adverse effects' increase, and dyskinesias are the most significant ones. There is evidence that SIBO contributes to the pathophysiology of motor fluctuations by decreasing the drug's bioavailability (18,30). And, by eradicating it, motor fluctuations improved without affecting the pharmacokinetics of levodopa. However, the relapse rate reached 43% as predisposing conditions were still there (30).

Recently there has been strong evidence about the benefits that probiotics use in PD patients can have, by improving constipation, increasing ghrelin levels (low in the vast majority of PD patients and related to delayed gastric emptying), decreasing oxidative damage by producing potential antioxidants and bioactive molecules, increasing the levels of the gut microbiota beneficial metabolites, and improving motor symptoms (32–34). However, there is no evidence about probiotics' benefits on the after-treatment of PD patients suffering from SIBO and how these can help prevent the relapse of motor fluctuations by improving the predisposing conditions.

Our study aims to provide more evidence on **whether probiotics as an add-on treatment in PD patients suffering from SIBO would decrease the relapse rate of this condition in the long term and thus, decrease the frequency and duration of motor fluctuations**. Therefore, we will design a randomized controlled trial to test the positive effects on avoiding SIBO relapse by treating these predisposing conditions with probiotics and how this can be related to motor fluctuations and levodopa bioavailability in the long term. In case of a positive outcome, it would be beneficial in clinical practice for multiple reasons. First, given that PD patients are, in most cases, polymedicated, probiotics seem to be a great option to treat constipation and delayed gastric emptying thanks to their low rate of adverse effects. On the other hand, this would avoid increasing the dose and frequency of levodopa in patients, helping to delay the appearance of dyskinesias. Therefore, this would positively impact patients' quality of life and public health expenses considering their reasonable price.

6. HYPOTHESIS:

Main hypothesis:

The use of probiotics as an add-on treatment to rifaximin in PD patients suffering from SIBO and motor fluctuations reduces SIBO relapse rate at 6 months.

Secondary hypothesis:

- The use of probiotics as an add-on treatment to rifaximin in PD patients suffering from SIBO results in an improvement in motor fluctuations in the long term.
- The use of probiotics as an add-on treatment to rifaximin in PD patients suffering from SIBO and motor fluctuations improves UPDRS-III scores during *ON*.
- The use of probiotics as an add-on treatment to rifaximin in PD patients suffering from SIBO and motor fluctuations improves the **Unified Dyskinesia Rating Scale** (UDysRS) during *ON* time.
- Levodopa bioavailability in PD patients suffering from SIBO and motor fluctuations who use probiotics increases or remains stable, thus, levodopa daily dose changes.
- Ghrelin levels increase by adding probiotics to PD's standard treatment and this improves gastric emptying.

7. OBJECTIVES:

Main objective:

To assess SIBO relapse rate at 6 months in PD patients SIBO+ and motor fluctuations who use probiotics as an add-on treatment to rifaximin.

Secondary objectives:

- To evaluate motor fluctuations improvement in the long term in PD patients suffering from SIBO and motor fluctuations who use probiotics as an add-on treatment to rifaximin.
- To rate UPDRS-III scores during *ON* time in PD patients suffering from SIBO and motor fluctuations who use probiotics as an add-on treatment to rifaximin.
- To value UDysRS during *ON* time in PD patients suffering from SIBO and motor fluctuations who use probiotics as an add-on treatment to rifaximin.
- To determine if probiotics use increases or maintains stable levodopa plasma levels in PD patients suffering from SIBO and motor fluctuations and to estimate the percentage of change in levodopa daily dose in patients needed by the study's end.
- To determine ghrelin levels in PD patients and its relationship with patients' gastric emptying.

8. METHODOLOGY:

8.1. Study design:

We designed a multicentre, randomized, double-blind, placebo-controlled, parallel-group study to assess how probiotics might influence SIBO relapse and motor fluctuations in PD patients who underwent the corresponding antibiotic treatment.

8.2. Study setting:

As this is a multicentre study, we consider as reference hospitals to participate in this study those hospitals in Catalonia that have a motor disorders department. The reference hospitals that will be participating in this study are:

Table 2. *Study Reference Hospitals*

Study Reference Hospitals			
Girona	**Barcelona**	**Lleida**	**Tarragona**
- Hospital Dr. Josep Trueta - Hospital Santa Caterina - Hospital de Figueres Fundació Salut Empordà	- Hospital Bellvitge - Hospital Clínic - Hospital General Sant Cugat - Hospital Parc Taulí - Hospital Sant Pau - Hospital Trias I Pujol - Hospital Vall d'Hebron	- Hospital Arnau de Vilanova - Hospital de Santa Maria de Lleida	- Hospital Joan XXIII - Hospital de Sant Joan de Reus

8.3. Study population:

The target population of this study will be individuals with a PD diagnosis. Neurologists from each reference neurology department will suggest to idiopathic PD-diagnosed patients the possibility of being included in the study as long as they meet the following criteria:

Inclusion Criteria:

- Male and female patients with idiopathic PD according to the UK-PDSBB criteria.
- Experiencing motor fluctuations while receiving levodopa and other dopaminergic treatments the last 6 months.
- Hoehn and Yahr stage II-III during *OFF* time.
- SIBO positive.
- Patients should be able to accurately keep a diary.
- Patients who accepted and signed the written informed consent (ANNEX Vand ANNEX IV).

Exclusion Criteria:

- PD patients with unpredictable or extreme motor fluctuations.
- Late-stage PD (Hoehn and Yahr IV).
- Patients with dementia, major psychiatric illnesses, or other severe medical illnesses.
- Any GI or systemic conditions that potentially affect GI motility.
- Immunocompromised patients.
- Previous exposure to proton pump inhibitors; immunosuppressive drugs; medications that affect GI motility such as prokinetics, anticholinergics, and tricyclic antidepressants; and antibiotics or any other medications that affect the intestinal flora such as laxatives in the past month.

Withdrawal Criteria:

- The patient has shown clinically significant deterioration under treatment.
- The patient requests to be excluded from the study.
- The patient does not participate in the follow-up interventions.
- The patient needs to go under oral antibiotic treatment for another reason.
- Death of the patient.

8.4. Sampling:

8.4.1. Sample size:

In a two-sided test, with an α =5% statistical power of 80%, and moderate effectiveness of the addition of probiotics to the rifaximin treatment, we will need 196 subjects per group. Assuming a drop-out rate of 10%, we will need finally 216 subjects per group, i.e., a total of 432 subjects.

Computations were carried out with Prof- Dr. Marc Saez' software based on the package 'pwr' of the free statistical environment R (version 4.2.1).

8.4.2. Time of recruitment:

The duration of patients' recruitment will be one year. Prior to enrolment, patients will have to sign the informed consent (ANNEX IV and ANNEX V). However, given the strict inclusion criteria, the time of recruitment can be adjusted according to needs until the sample size is entirely achieved.

8.4.3. Methods of recruitment:

The distribution of the sample (n=432) between the different hospitals will be proportional to the number of patients in each hospital.

We will be conducting a consecutive non-probabilistic sampling method, including outpatients and inpatients meeting inclusion and exclusion criteria in the referral hospitals mentioned above.

To improve patients' participation, it is essential to deliver insights into our study, hence the importance of an accessible informed consent (ANNEX IV and ANNEX V), as well as a re-explanation of the study plan in an understandable way.

8.4.4. Randomization and masking:

Randomization will be performed using a computer-generated randomization method and the study will be double-blinded. The patients and the clinical practitioners who distribute the treatment will be blinded as to the treatments assigned. The patients entering the study will be randomised into one of the two following groups:

- **Active treatment group:** Patients receiving probiotic supplements after SIBO treatment.
- **Placebo group:** Patients who will be receiving supplements of similar appearance to the probiotics used but without the microbial cells, that is, placebo.

8.5. Variables and measurement methods:

Independent Variable:

- **Administration of probiotics:** For our study, we will use a probiotic with the following composition: *L. acidophilus* SD5212 (DSM 24735), *L. casei subsp. paracasei* SD5218 (DSM 24733), *L. delbrueckii subsp. bulgaricus* SD5210 (DSM 24734), *L. plantarum* SD5209 (DSM 24730), *B. longum* SD5219 (DSM 24736), *B. infantis* SD5220 (DSM 24737), *B. breve* SD5206 (DSM 24732), *S. thermophilus* SD5207 (DSM 24731) under the brand name VIVOMIXX from the Grifols company. This probiotic comes in capsules at 1,15 X 10^{11} CFU or sachets at 4,5 X 10^{11} CFU. It will be a dichotomous qualitative variable expressed as yes or no, depending on whether the patient is taking a probiotic or placebo.

Dependent Variable:

- **Main dependent variable:**

  - SIBO presence: It will be a dichotomous qualitative variable expressed as yes or no. The **lactulose breath test** (LBT) and **glucose breath test** (GBT) will be used to assess the presence of SIBO. The test will be considered positive if H_2 levels are $\geq$ 20 ppm by 90 minutes in the GBT and LBT test.

- **Secondary dependent variables:**

  - Presence of motor fluctuations: Through the variables daily *ON* time (sum of *ON* time without dyskinesia and *ON* time with nontroublesome dyskinesia), daily *OFF* time, asleep, wearing-*OFF* and time to *ON*. These will be a quantitative continuous variable that shall be measured in hours. Patients will complete a daily diary (35) to record this data, and proper training should be provided to reach a concordance between the patients' and investigators' ratings on each term's meaning.

  - Motor symptoms during *ON:* Trained physicians will evaluate scores during *ON* time. It is a quantitative discrete variable since UPDRS-III (motor) can only take a finite number of real values in a specific interval.

  - Dyskinesia during *ON:* UDysRS will be used and evaluated by trained physicians. It is a quantitative discrete variable.

  - Levodopa plasma levels: Peak plasma concentration of dopamine will be calculated after 1,5h of levodopa intake to study levodopa plasma levels, and will be measured at each study visit after levodopa intake. It will be a quantitative discrete variable.

  - Levodopa daily dose: From T1 to T2, changes in levodopa daily doses will be allowed and registered. This is a quantitative continuous variable. It is important to rate the changes in levodopa dose.

  - Ghrelin plasma levels: Fasting blood samples will be collected and sent at the reference laboratory. It is a quantitative discrete variable. Units of measure will be pg/ml.

Table 3. *Summary of variables assigned in the study.*

	VARIABLE	DESCRIPTION	CATEGORIES	MEASUREMENT
Independent Variable	Probiotics supplementation	Dichotomous qualitative	Yes/No	Investigator report
Main Dependent Variable	SIBO presence	Dichotomous qualitative	Yes(>20ppm)/No	LBT and GBT
Secondary Dependent Variables	Motor fluctuations: Daily *ON*, delayed-*ON*, no-*ON*, Daily *OFF*, and wearing-*OFF*.	Quantitative continuous	Time in hours	Patients' diary Levodopa challenge
	UPDRS-III during *ON*	Quantitative discrete	0-68	UPDRS-III(motor)
	Dyskinesia during *ON*	Quantitative discrete	0-73	UDysRS
	Levodopa plasma levels	Quantitative continuous		Laboratory
	Levodopa daily dose	Quantitative continuous	mg/day	Neurologist report
	Ghrelin plasma levels	Quantitative continuous	Lower and upper limits are 0-650 pg/mL	Radioimmunoassay by the reference laboratory

Covariates:

The following variables may play an important role in modifying the results attributable to their influence on the course of PD. Therefore, they must be taken into account when analysing the results.

- **Age**: It is a quantitative continuous variable expressed in years. Typically, the older the patient is, the more advanced the PD stage is, thus, more neurodegeneration present.

- **Sex**: It is a nominal variable expressed as male or female. There have been some differences in clinical manifestations between males and females.

- **Ethnicity:** It is a qualitative polytomous variable. Related to differences in diet and metabolism.

- **Body Mass Index (BMI):** It is a quantitative discrete variable expressed as kg/m^2. Low body weight has been related to a higher risk of dyskinesias.

- **Hoehn and Yahr Stage (H&Y):** Grouped into 4 different stages. Qualitative polytomous variable.

- **Time from diagnostic:** It is a quantitative continuous variable. It is important to consider time since diagnosis because the extent to which the probiotics might be beneficial may differ depending on the stage of the disease.

- **Levodopa treatment duration:** It is a quantitative continuous variable expressed in years. More severe motor fluctuations can be related to a more extended period of levodopa exposure.

- **Concomitant PD medications:** Dopamine agonists, COMPT inhibitors, MAO-B inhibitors, amantadine, apomorphine… Results could be influenced by the levodopa concomitant treatments the patient is taking. It is a qualitative polytomous variable.

- **Concomitant *Helicobacter Pylori* (HB) infection:** There is evidence that HB infection can also influence levodopa bioavailability (36). Qualitative dichotomic variable.

- **Socioeconomic status:** Assessed by the level of education attained. Patients must be able to keep a diary. Those with a lower socioeconomic status are more likely to enter the data incorrectly. It is a polytomous variable.

- **Hospital patients come from.** There might be differences in PD patients' management. Qualitative polytomous variable.

Table 4. *Covariates assigned in this study.*

	VARIABLE	DESCRIPTION	CATEGORIES	MEASUREMENT
Covariates	Age	Quantitative continuous		Anamnesis
	Sex	Qualitative dichotomous	-F: Female -M: Male	Anamnesis
	Ethnicity	Qualitative polytomous		Anamnesis
	BMI	Quantitative discrete	Kg/m^2	Physical examination
	H&Y	Qualitative polytomous	8 stages	Physical examination
	Time from diagnosis	Quantitative continuous	Years	Medical history
	Levodopa treatment duration	Quantitative continuous	Years	Medical history
	Concomitant PD medications	Qualitative polytomous	Report the name	Medical history
	Concomitant HB infection	Qualitative dichotomous	Yes/No	Urea test
	Socioeconomic status	Polytomous variable	Low, medium, or high	Anamnesis
	Hospital patients come from	Polytomous variable	**Table 2.** Study Reference Hospitals	Medical history

8.6. Study intervention:

To conduct our study, PD patients suffering from motor fluctuations and meeting our criteria (without regard to whether they are SIBO -positive or -negative) will be selected by our neurologists at our referral centres. After being informed about the study and once they have signed the consent form for participation (ANNEX IV and ANNEX V), they will undergo a 3-day levodopa stabilization phase. It will involve the patient keeping a diary, completing information about levodopa's effect that we aim to study (**daily *ON* time** (sum of *ON* time without dyskinesia and *ON* time with nontroublesome dyskinesia), **daily *OFF* time**, and **asleep**). The diary will be completed at 30-minute intervals for 18 hours for 3 days after taking their usual medication. The patients' diaries will be evaluated by their neurologists to ensure that it is properly completed. Before the stabilisation phase begins, there must be agreement between the patients' assessments and those of the investigators.

After concluding with the levodopa stabilization phase, patients will be tested for SIBO. LBT and GBT will be used to assess the presence of SIBO. Patients would be classified as SIBO positive (+) if at least one of the two methods was positive.

If PD patients test positive for SIBO, a **baseline visit** shall be scheduled to evaluate the following:

- Personal data: We aim to record information about the patients' age, sex, ethnicity, and socioeconomic status.
- Current data: Weight, height, BMI, HB infection (through a urea test).
- PD-related data:
    - **General data:** Time since diagnosis, H&Y stage, PD treatment (levodopa and other drugs), and daily levodopa intake.
    - **GI symptoms:** The following conditions will also be recorded: pain, flatulence, belching, nausea, vomiting, constipation, diarrhoea, loss of appetite (37).
    - **UPDRS-III at *ON:*** A trained physician will evaluate the patients' motor performance at the *ON* phase.
    - **UDyRS at *ON*:** A trained physician will evaluate the appearance of dyskinesia at the *ON* phase.

- **Levodopa plasmatic levels:** Peak plasma concentration of dopamine will be calculated after 1,5h of levodopa intake.
- **Ghrelin plasma levels:** Fasting blood samples will be collected and evaluated.

After SIBO diagnosis, and when the pre-evaluation is done, patients will go through the following phases:

Phase 1:

All SIBO+ patients will be treated with rifaximin 400 mg/3 times daily for 14 days. A previous study (30) treated patients for 7 days, and eradication in all subjects was not achieved. Therefore, we decided to extend the treatment for another week according to other recommendations (18). From the time the patient is diagnosed as SIBO+ until the end of antibiotic therapy, the PD treatment will be kept constant.

Phase 2:

Patients will be evaluated one month after antibiotic treatment to determine if SIBO has been successfully eradicated. In addition, patients who become SIBO- will be re-examined for the aforementioned PD-related data. That is, weight, BMI, GI symptoms, UPDRS-III at *ON*, UDyRS at *ON*, and levodopa plasma levels. We do not consider it necessary to evaluate ghrelin levels at this time. The patients' diary will also be completed in the 3 days prior to the study visit.

Phase 3:

Patients will be randomized in two groups. Those taking probiotics, and those receiving placebo.

For our study, we will use a probiotic with the following composition: *L. acidophilus* SD5212 (DSM 24735), *L. casei subsp. paracasei* SD5218 (DSM 24733), *L. delbrueckii subsp. bulgaricus* SD5210 (DSM 24734), *L. plantarum* SD5209 (DSM 24730), *B. longum* SD5219 (DSM 24736), *B. infantis* SD5220 (DSM 24737), *B. breve* SD5206 (DSM 24732), *S. thermophilus* SD5207 (DSM 24731) under the brand name VIVOMIXX from the Grifols company. This probiotic comes in capsules at 1,15 X 10^{11} CFU or sachets at 4,5 X 10^{11} CFU.

PD patients often suffer from dysphagia; thus, we decided that the best option is for them to take capsules instead of sachets. Patients will be instructed to consume one capsule daily.

The market has a wide range of probiotic types; we selected this brand because of it is readily available and because its composition was recommended by the Spanish consensus on probiotics in IBS and constipation cases, which have the same clinical symptoms as PD (27).

Patients in the control group will take a placebo, administered in the same way as the probiotic preparation, and the capsule will also have a similar appearance. Patients will be instructed to consume one capsule daily. The placebo will be prepared by the hospital pharmacists, and they will be the only ones who will know about the composition of the capsules.

Patients will be taking probiotics or placebo for 4 months. The benefits of probiotics depend on each medical condition; it takes around 3 months for some patients to notice the effects (27). Therefore, we decided that our patients would undergo a 4-month probiotic therapy to ensure they have sufficient time to restore the gut microbiota and to benefit from their metabolites. From this point, changes in levodopa treatment can be made, but the adjustments must be recorded to establish the percentage of change in levodopa dose. On the other hand, other medications cannot be added to avoid confounding factors.

Phase 4:

Once the four months have elapsed, patients will stay without treatment for a month. The reason behind this decision is that, in order to have a GBT and LBT with a meaningful outcome, probiotics should be stopped 1 month prior the evaluation (18).

Phase 5:

Another study visit will take place. Again, 3 days prior, patients' must complete their daily diary. In addition, we will perform another GBT, and LBT test, check BMI, record GI symptoms, UPDRS-III at *ON*, and UDyRS. Finally, levodopa and ghrelin plasmatic levels will be evaluated as well.

8.7. Measure instruments:

Patients' diaries:

Patients' diaries assess essential variables related to PD clinical manifestations while on treatment, allowing a better understanding of their effect on PD patients, and a better evaluation of the clinical intervention. More diary days provide greater reliability, but evidence has shown that reliability is reduced after three days because more errors occur (35). The functional status designations on the diary we will be using are ASLEEP, *ON* time (as the sum of *ON* without dyskinesia and *ON* with nontroublesome dyskinesia), and *OFF*. Each one of these parameters will be measured in hours.

Hydrogen breath test:

The hydrogen breath test is a non-invasive method to diagnose SIBO. Microorganisms in the intestine metabolize carbohydrates into gases that can later be evaluated. These gases pass into the bloodstream and are exhaled. There are two types of hydrogen breath tests according to what kind of sugar the patient takes, the LBT and the GBT. Their sensitivity and specificity are lower compared to culture from jejunal aspirate, which is regarded as the main gold standard for SIBO diagnosis. However, the latter has several methodological limitations; it is invasive, resource-consuming, less available, has a higher risk of oral flora contamination, and if areas that are not colonized are aspired, there is an important risk of false negatives. On the other hand, the GBT can be used to evaluate the presence of SIBO in the first part of the small intestine; and the LBT can be used to assess SIBO in the distal small intestine. We aim to achieve greater diagnostic accuracy by combining these two techniques. The GBT and LBT tests will be considered positive if H2 levels are $\geq$ 20 ppm by 90 minutes. Patients will be asked to fast for 12 hours before the test. After that, the test will be performed, and it will take around 3 hours. Human cells are not capable of producing hydrogen gas or methane gas, so their presence means that fermentation of this substrate (glucose or lactulose) has occurred in the intestine. When excessive amounts of gas are produced, they penetrate the intestinal walls and are carried through the bloodstream to the lungs, where they are excreted. Only then can we detect and quantify them with the instrument (18).

UPDRS-III (motor):

The UPDRS has four parts: Part I (non-motor experiences of daily living), Part II (motor experiences of daily living), Part III (motor examination), and part IV (motor complications). In our trial, we will be using Part III (ANNEX II) to assess motor signs of PD. Evaluations will be performed at *ON* state after the patient receives his usual medication and responds well (38).

Items that will be evaluated are speech, facial expression, rigidity, finger tapping, hand movements, pronation-supination movements of hands, toe-tapping, leg agility, arising from a chair, gait, freezing of gait, postural stability, posture, body bradykinesia, postural tremor of the hands, kinetic tremor of the hands, rest tremor amplitude, constancy of rest tremor, dyskinesia impact on part III ratings. All items must have an integer rating. This section has a possible range of scores of 0-68 points; the higher the number, the more severe the patients' condition is.

UDysRS:

The **Unified Dyskinesia Rating Scale** (UDysRS) (ANNEX III.) will evaluate involuntary movements associated with PDs. There are two primary sections, the *ON*-dyskinesia section, assessing choreic and dystonic movements when the medicine is working, and *OFF*-dystonia, assessing spasms or cramps that occur when PD's medications are not taken or are not working. It measures dyskinesias in different body areas, the degree of impairment caused by them when patients perform daily living tasks and the patients' perception of disability from dyskinesias (39).

Levodopa plasma levels:

Levodopa plasma levels will be determined by a catecholamine blood test, in which the peak dopamine level is determined 1.5 hours after levodopa intake. A blood sample will be required to measure dopamine concentration, and a dopamine ELISA kit, a specific enzyme immunoassay, will be used. Both extraction and quantification procedures will be carried out on the same day by our Reference Laboratory.

Ghrelin plasma levels:

Ghrelin is an orexigenic hormone produced in the stomach. Its levels increase during periods of low food intake, such as fasting, starvation, and anorexia. Receptors in the stomach stimulate ghrelin production when the stomach is empty, and stop production when they sense that the stomach is being stretched. Ghrelin acts on the nervous system to stimulate appetite and reward pathways after food intake. Like all hormones, once it is produced, it circulates in blood. Therefore, fasting blood samples will be required to determine ghrelin plasma levels (0-650 pg/mL) using a specific enzyme immunoassay in our Reference Laboratory.

8.8. Flowchart:

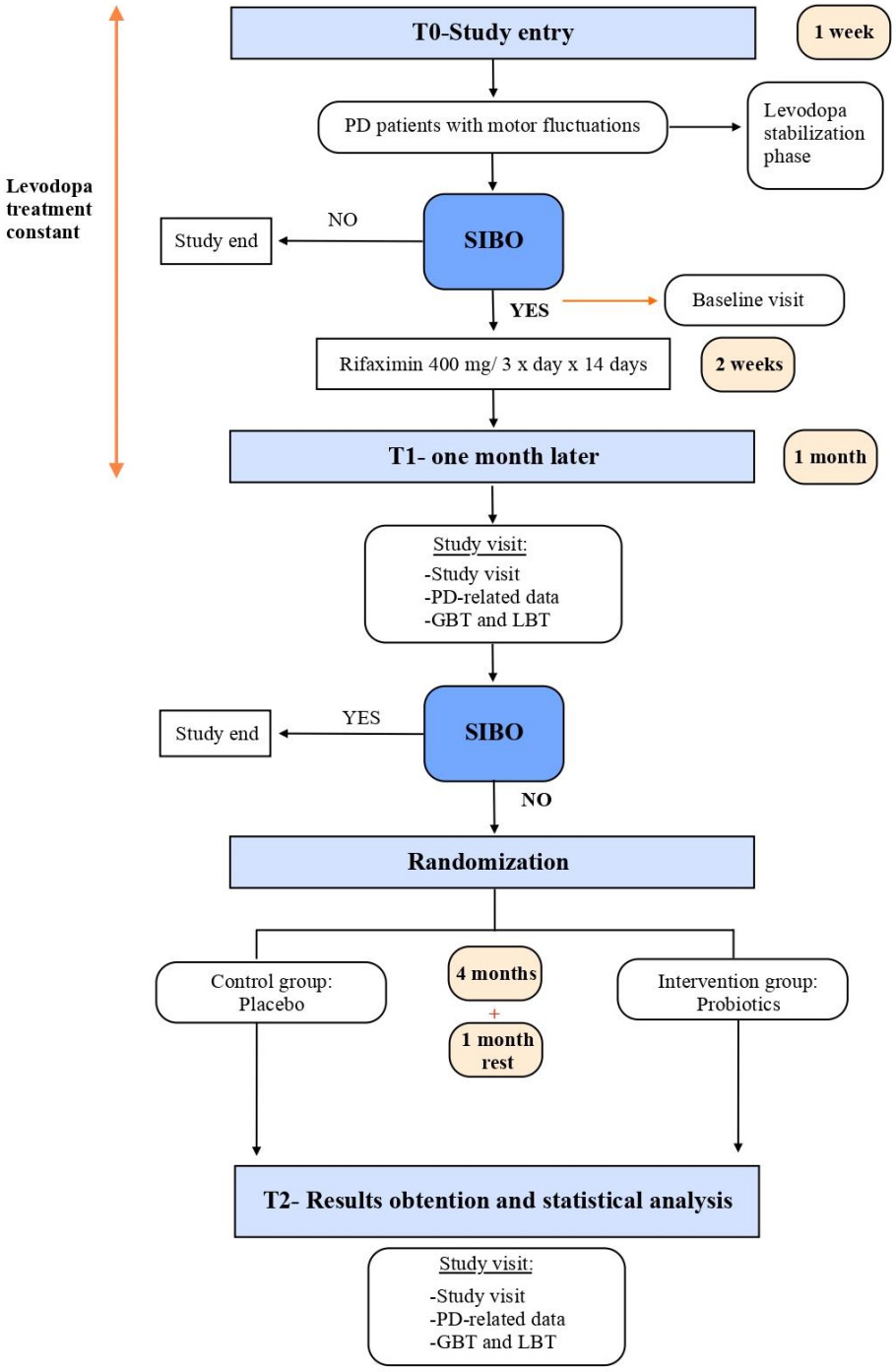

Figure 6. *Study flowchart.*

PD: Parkinson's disease; **SIBO**: Small intestine bacterial overgrowth; **GBT**: Glucose breath test; **LBT**: Lactulose breath test

8.9. Safety:

Probiotics are living non-pathogenic microorganisms that act as a supplement to restore gut microbiota, thus, recovering its normal function. To bring a successful probiotic to market, it takes several steps to choose the proper stain and ensure it is safe. Probiotics' adverse effects can be classified into 5 types:

- Infectivity or pathogenicity
- Production of non-desirable metabolites
- Possibility of antibiotic resistance genes transmission
- Excessive immunostimulation or immunodepression in sensitized individuals
- Side effects related to the excipients

To date, most genera are *Lactobacillus* and *Bifidobacterium* since no genes related to pathogenicity have been described yet. Therefore, the use of probiotics as a supplement is typically safe. Cases in which a relationship between probiotic consumption and adverse effects has been established are extremely rare. And although we decided to remove immunocompromised patients from our study, clinical trials in which probiotics have been administered to immunocompromised hosts have confirmed their low pathogenicity.

9. STATISTICAL ANALYSIS:

The statistical analysis will be performed by the statistician, using IBM SPSS Statistics version 28.0.1.1. We will consider results to be statistically significant if p-values < 0,05, and the confidence intervals will be expressed as 95%.

9.1. Descriptive analysis:

We will perform a descriptive analysis for all variables, which will vary depending on whether they are qualitative or quantitative:

- SIBO presence, a qualitative variable, will be summarized as a percentage $\pm$ confidence interval (95%).
- The quantitative continuous variables, motor fluctuation, levodopa plasma levels, levodopa daily dose, and ghrelin plasma levels will be summarized as mean $\pm$ standard deviation (SD), and median and interquartile range [Q1, Q3].
- The quantitative discrete variables, UPDRS-III and dyskinesia during *ON* will be summarized as median and interquartile range [Q1, Q3].

These analyses will be stratified by intervention and control group and, additionally by the covariates. Quantitative covariates will be categorized. BMI will be categorized in normoweight (<25 kg/m^2), overweight (25-30 kg/m^2) and obese (>=30 kg/m^2). The rest of quantitative covariates will be categorized in quartiles.

9.2. Bivariate inference:

The difference of proportions of SIBO presence between the intervention and the control group will be tested by Chi-squared or the Fisher's exact test (if the expected number of cases will be lower than 5). The difference of means between the addition probiotics or placebo will be tested using the Student's T test. Finally, the difference of medians will be tested through the Mann-Whitney's U test.

These tests will be stratified by the covariates.

9.3. Multivariate analysis:

To assess the effects of the intervention on the different variables will be adjusted regressions controlling for the covariates. The type of the regression depends on the type of the dependent variable:

- Qualitative dichotomous dependent variable, SIBO presence: logistic regression.
- Quantitative continuous dependent variable: motor fluctuation, levodopa plasma levels, levodopa daily doses, and ghrelin plasma levels: linear regression.
- Quantitative discrete variables, UPDRS-III and dyskinesia during *ON*: Poisson regression.

10. WORKING PLAN:

Our research team will include a **main investigator** (MI), a **neurologist** in each reference centre (NG) as co-investigator, hospital staff, a **project manager** (PM), and a **statistician** (ST). At each centre, the **neurologists** will help recruit patients, the **nurses** will help perform the tests, and the **pharmacologists** will help by crafting the placebo capsules. This clinical trial will have an approximate duration of 3 years, in accordance to the activities organized in the following phases:

Stage 1. Study design, preparation and coordination:

This phase will last 6 months, from January 2023 – June 2023

- **Study design:** The MI will conduct an extensive literature research to determine the main hypotheses and objectives of the study. Based on this information, a research protocol will be developed, including a detailed explanation of the variables and objectives submitted for the study and the definition of the analytical framework. A PM will be recruited to administer the information on the patients, manage the collection of variables, ensure the deadlines are met, and be responsible for proper communication between all the professionals involved. This step will take around 2 months.
- **Centres and Ethical Committee approval:** The reference hospitals selected as potential members of this trial will be contacted to ask for their involvement. Each centre will have a representative for the neurology department, and will be responsible for informing the patients about the possibility to participate in this study. The protocol will be submitted to the ***"Comité de Ética e Investigación Clínica"*** (CEIC) for revision and approval in each centre. All proposed amendments will be taken into account. This process could take about 3 months.
- **Coordination meetings:** This step consists of organizing meetings with the whole team to define the tasks of each individual. The team will mainly consist of the MI, the NG in charge of each neurological department at the reference centres, and the PM. The neurologists will be given the information they must to convey to the participants. The team altogether will also draw up the chronogram. During the first phase, the researchers will meet regularly to monitor the study's progress. A workshop will also be recognized to determine a common method of datal collection. This phase will take around 1 month.

Stage 2. Sample collection, intervention, and data collection:

This phase will last 2 years and a month, from July 2023 – August 2025.

- **Sample collection:** Patients who meet the inclusion and exclusion criteria will be invited by their neurologists to our study using a consecutive sampling procedure. The NG responsible for the project in each hospital will explain the study in more detail, and patients will be asked to provide informed consent (ANNEX IV and ANNEX V). This process will take one year.
- **Baseline data collection:** Patients eligible for the study who have signed a consent form will be visited at baseline (T0) to collect current personal data and PD-related information. In addition, patients will complete a diary for 3 days to record key variables related to PD clinical manifestations during treatment and assess motor fluctuations. This step will take approximately 1 week for each patient. Neurologist visits will take approximately 1 hour, and to determine if they are SIBO positive or negative, an additional 3 hours will be required. Laboratory determinations to determine peal plasma dopamine levels will require 1.5 hour wait. It is recommended to start with the blood samples when the ghrelin levels need to be checked (T0 and T2). Later, the presence of SIBO will be tested; finally, the neurologist will visit the patient after taking medication. After that, another blood sample will be needed to check the dopamine levels. For this reason, the patient will need to stay in the day hospital, our visits will last about 5 hours in total.
- **Intervention and follow-up:** Once SIBO is diagnosed by the nurses, patients will be treated and randomized into two groups, those taking placebo (elaborated by the hospital pharmacologists) and those taking probiotics. Data will be collected at T1 and T2 during study visits. All patients will be required to complete the diary 3 days prior to each study visit, and further information will be collected during the study. This step will take seven and a half months and will be carried out by the neurologist responsible for the study in each hospital.
- **Data generation:** Once patients have participated in the intervention, the PM will enter the information collected into the database. This process will take 1 month.

Stage 3. Statistical analysis:

This phase will last 4 months, from September 2025 – December 2025.

- **Statistical analysis:** To be conducted by a masked ST at the end of the study. The duration of this step will be of one month.
- **Evaluation:** This step will be carried out by the MI and the ST. It will take a month.
- **Final report phase**: The research team will meet to discuss the results and prepare a final report with the study findings and conclusions. The MI will take responsibility for this step. This phase will take 2 months.

Stage 4. Publication of results:

This phase will last 2 months, from January 2026 – February 2026.

- **Paper publication:** The clinical trial results will be published as soon as the paper is finished. It will be sent to medical journals.
- **Dissemination:** The final report will be presented at congresses.

Table 5. *Schedule of assessments*

Stage	Assessments	T0		T1	T2
		1st week	2nd week		
Recruitment	Informed consent	X			
	Baseline visit	X			
	Hydrogen breath test	X		X	X
Intervention	Rifaximin		X		
	Randomization			X	
	Probiotics/Placebo			X	
PD-related data	General data	X		X	X
	Patients' diaries	X		X	X
	GI symptoms	X		X	X
	UPDRS-III at *ON*	X		X	X
	UDyRS at *ON*	X		X	X
	Levodopa plasmatic levels	X		X	X
	Ghrelin levels	X			X

11. CHRONOGRAM:

Stages	STAFF	2023												2024												2025												2026	
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F
Stage 1. Study design, preparation, and coordination																																							
Study design	MI																																						
Centres' and ethical committee approval	MI, CEIC																																						
Coordination meetings	MI, NG																																						
Stage 2. Sample collection, intervention, and data collection																																							
Sample collection	NG,																																						
Baseline data collection	NG, nurses																																						
Intervention and follow-up	NG, nurses, PH																																						
Data generation	PM																																						
Stage 3. Statistical analysis																																							
Statistical analysis	ST																																						
Evaluation	ST, MI																																						
Final report phase	MI																																						
Stage 4. Publication of results																																							
Paper publication	MI																																						
Diffusion	MI																																						

12. ETHICAL ASPECTS:

The protocol will be submitted, reviewed, and evaluated by the local Clinical Research Ethics Committee (CEIC) of each of the reference hospitals in our multicentre study; they will ensure that the protocol meets the ethical requirements for approval. Managements' Department authorization of all the medical centres participation in the study will also be required.

This study will be conducted according to human rights and the ethical principles for medical research described by the ***Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects*****,** established in 1964 and last revised in October 2013, and the *Principles of Biomedical Ethics* from *Beauchamp and Childress* from 1970 and reviewed in 2009:

- Autonomy: The values and personal choices made by participants will be respected throughout the study. All patients will be appropriately informed about the study's aims before participating in the trial. An information sheet (ANNEX IV and ANNEX V) will be handed out and adequately explained to patients in order for them to understand the information before being asked to sign the consent form (ANNEX IV and ANNEX V) to participate in the trial, always respecting their autonomy.

- Non-maleficence: No malicious intent is intended, and patients will be excluded from the study if our intervention shows a clinically significant worsening.

- Beneficence: All our interventions will be performed with the primary aim to benefit the patient by using a reasonably harmless intervention, probiotics, to improve levodopa bioavailability, and consequently, improve *ON* time and quality of life in PD patients suffering from motor fluctuations.

- Justice: Discrimination in access to our trial against any group of people will be prevented to ensure equity. In addition, patients will not receive any compensation for their participation.

The development of this protocol will also follow the guidelines set out in ***"The Real Decreto 1090/2015, de 4 de diciembre, por el que se regulan los ensayos clínicos con medicamentos, los Comités de Ética de la investigación con medicamentos y el Registro Español de Estudios Clínicos."***

To guarantee anonymity and confidentiality while processing patients personal data required in this study, our procedure will follow:

- The ***Regulation (EU) 2016/679 of the 27th April of 2016 European Parliament and Council, on people protection regarding the processing of personal data and free flow data***
- The repealing *Directive 95/46/EC (General data Protection Regulation)*
- The ***"Ley orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los Derechos Digitales (LOPD-GDD).***
- The ***"Real Decreto 1720/2007, de 21 de diciembre por el que se aprueba el Reglamento de desarrollo de la Ley Orgánica 15/1999).***

This study offers anonymity to patients by identifying them in the database with a code number. Access will only be possible for the research team and only for study purposes.

Researchers declare they have no conflict of interest. The primary aim of this trial is to provide generalizable knowledge to improve clinical management and patient quality of life. Furthermore, researchers agree to issue all data results in full, including unfavourable results.

13. BUDGET:

In order to calculate the required budget for this study, we have divided the costs into different sections:

Staff costs:

The budget does not include the cost of the main investigator, neurologists, nurses, and hospital pharmacologists, as the hospitals employ them. Each patient will be visited 3 times during the course of our study. This procedure will have no cost. In addition, we will need to hire a project manager to carry and enter all the information collected into the database and coordinate the different teams. We estimate that this will take 150 hours, which will cost 40€/hour. Finally, we also need the help of a statistical analyst whose work will take 90 hours, at 40€ each.

Expenses for the research teams' meetings will need to be considered. A total of 2 meetings will be held. Therefore, transportation and meals will incur costs of about 300 €.

Material expenses:

For the intervention group, we will use probiotics as an additional treatment. The brand we have chosen sells the product in boxes of 10 capsules. Each box costs 11,21 €. Each patient will need one capsule per day for 4 months. Therefore, we need a total of 12 packs for each patient. This corresponds to a total amount of 29.056,32 €. For the placebo group, we also need capsules with similar appearance. These will be manufactured by the hospitals' pharmacologists and will have an approximate cost of 6000 €.

Laboratory tests will be required to determine levodopa and ghrelin plasma levels. Patients will be tested three times for levodopa, and twice for ghrelin. The levodopa plasma levels will be measured by *Reference Laboratory*, the cost of each determination will be around 95,85 €. Ghrelin plasma levels will be measured by the same laboratory, at a cost of 75,85 € for each determination.

Patients will undergo a hydrogen breath testing three times. Each hydrogen breath test has a cost of 25€, for 432 patients, resulting in a cost of approximately 64.800 €. In addition, we need to consider the urea breath test to assess the presence of HB; its cost is about 15 €.

Printing expenses:

This includes the documents that need to be printed for our study, such as information documents, consent forms, and questionnaires. The approximate cost is about 200€.

Publishing expenses:

Once the study is completed, the results will be published. A total of two publications will be made at a cost of approximately 5000 €.

Travel expenses:

Dissemination activities will also be required after the publication of the study. Registration fees and transportation costs will be covered for the MI to attend a national congress, the total cost of which will be 1000€ per person, and an international congress, for which approximately 2000 € will be incurred.

The costs may seem high. However, it should be made clear that the main reason of this excessive cost is because of the laboratory tests that we ask for to prove our hypotheses. If the results were to be positive, and confirmed our hypotheses, the laboratory tests would not be necessary. And the clinical applicability would actually be quite high. It is important to point out that a hydrogen breath test and four months of probiotic treatment would be less costly in the long run than increasing levodopa intake or taking new medications. Therefore, the impact on patients' quality of life and the economic benefits for our Healthcare System by reducing these expenditures are of great importance. Therefore, we planned to apply for a grant from the *Fondo de Investigaciones Sanitarias del Instituto de Salud Carlos III* to cover these costs.

Table 6. *Budget.*

Item		Amount	Price	Subtotal
Staff costs				
Neurologists		-	0€	**0 €**
Nurses		-	0€	**0 €**
Laboratory team		-	0€	**0 €**
Hospital pharmacologists		-	0€	**0 €**
Main investigator		-	0€	**0 €**
Project manager		150h	40€/h	**6000 €**
Statistical analyst		90h	40€/h	**3600 €**
Coordination meetings (transportation and meals)				**300 €**
Material expenses				
Probiotics supplementation		12 packs per patient (n=216)	11,21 €/pack	**29.056,32 €**
Placebo		12 packs per patient (n=216)		**6000 €**
Laboratory testing	Levodopa plasma levels	3 times/patient	95,85 €/ determination	**124.221,6 €**
	Ghrelin plasma levels	3 times/patient	75.85 €/ determination	**65.534,4 €**
Hydrogen breath test		1296 times	25€/test	**32.400 €**
Urea breath test		432 times	15€/test	**6480 €**
Printing expenses				
Documentation			0,03€/page	**60€**
Publishing expenses				
Open Access				**2000€**
Another journal				**1000€**
Presentation expenses				
Conference registration, traveling, accommodation, and meals		1 researcher	Nationally: 1000€/p Internationally: 2000€/p	**3000€**
TOTAL				**279.652,32€**

14. STRENGTHS AND LIMITATIONS OF THE STUDY:

14.1. Strengths:

- Significant results will have a great impact on the healthcare system. For one, by avoiding SIBO relapse, levodopa's pharmacokinetics will be less impaired. In the long term, this would benefit the patients' quality of life by delaying the onset of severe motor fluctuations. In addition, it would represent an economic benefit because we could improve levodopa's bioavailability by adding a much less expensive probiotic instead of increasing levodopa's dose and frequency, which would also increase the risk of dyskinesias in PD patients. On the other hand, not only motor symptoms will be less severe, but also the NMS, also very important in PD patients. Finally, these findings could lead to more research on gut microbiome restoration in PD patients in order to demonstrate causality rather than association or correlation. Gut microbiota can be a new approach for a more personalized management of PD patients.

- Positive results could provide an opportunity to establish SIBO assessment as part of the general protocol for newly PD-diagnosed patients management. This would help prevent the occurrence of peripheral caused motor fluctuations.

- Even if probiotics are ineffective in preventing SIBO relapse, at least all patients will benefit from their treatment.

- This is an easy study to perform, with a practical applicability. If results turn out to be positive, it would be an inexpensive intervention for daily practice.

- This is a double-blind trial. Thus, we minimize bias and placebo effect.

- This is a randomized clinical trial, which means that meaningful results will allow us to conclude if this intervention has a significant impact in PD patients SIBO+ suffering from motor fluctuations.

14.2. Limitations:

- We will be conducting a clinical trial. By its very nature, it will be an expensive study. Procedures that are not routinely performed in the day-to-day practice of a PD patient will be conducted, and their cost is high. On the other hand, if the results turn out to be positive, it would be economically beneficial in the long term.

- There might be inter-hospital variability between UPDRS and UDysRS ratings. To reduce variability, meetings will be held to establish concordance between the different professionals.

- Although rare, probiotics may present adverse effects. This increases the risk of patient withdrawal from the trial. However, we anticipate that dropout rates to be low. On the other hand, if this were the case, we would be able to finish outlining probiotics adverse effects in PD patients.

- PD patients in their late stages and patients suffering from other severe illnesses will be excluded from this trial. Because of this reason, the possibility of extrapolating the results to the general PD population is difficult. Besides, given the complexity of PD's pathophysiology and the different forms of the disease, modification of the disease's course by a single intervention is unlikely.

- The probiotic combination we used makes it almost impossible to compare our results with other studies. We aimed to restore PD patients gut microbiota without a proper metagenomic study prior to it, nor after our intervention. This a possible area of improvement.

- No dietary factors controls have been performed. Diet depends on geographical location, ethnicity, accessibility, and habits. And it is an essential factor when talking about gut microbiota. The lack of information about the dietary habits of our patients is an important limitation of our study.

- Given the strict enrolment criteria, the population study is extremely selected, and it is difficult to extend the conclusion to all PD patients.

15. FEASEABILITY AND IMPACT ON THE HEALTH CARE SYSTEM:

15.1. Feasibility:

This multicentre clinical trial will be conducted in fourteen hospitals in Catalonia to ensure that we reach the required sample (n=432). We expect to recruit patients after one year, since PD is the second most common neurodegenerative disease and its prevalence is quite high. The duration of the study will be long enough to test our main hypothesis and obtain a representative result. Because patient participation in the study is relatively short (7,5 months), we anticipate that the dropout rates will be low and that a good follow-up will be conducted.

Their neurologists will be the ones responsible for talking adequately to these patients about the possibility of participating in our clinical trial. And since our clinical trial is an intervention that could increase the benefit of standard PD medications, patients are likely to participate. The medical team needed for this study consists of a neurologist in each centre, who will collect all PD-related data and evaluate the scales, and the other professionals who are already part of the hospital team that and will collaborate on other aspects of our study (hydrogen breath test, blood samples, manufacturing placebo). They all belong to the hospitals; therefore, they will not increase the healthcare costs. On the other hand, a project manager and a statistical analyst will be needed, both of whom will receive economic compensation. We will hold informational meetings with all researchers and staff who will participate in the study to explain and clarify the required aspects and to ensure that the differences between hospitals are as small as possible. The blood and breath tests that will be done on patients will add significantly to the cost of our studies. But they will be done only to prove our hypotheses. If the results are positive, blood tests will longer be necessary, which will significantly reduce the cost to the health care system and facilitate the applicability of the procedure. In addition, the hospitals we selected are able to perform these or send them to the Reference Laboratory through the ICS at public system price. The antibiotics used are available in all our hospitals, and the probiotics used in this study are also available and affordable, and no major adverse effects have been recorded, facilitating their use.

Therefore, we believe that this study meets all the requirements to be conducted considering the study sites, economic costs, and the number of patients needed.

15.2. Impact on the health care system:

PD is the second most common neurodegenerative disease in the world. Although it is known for its motor symptoms, PD patients suffer from a variety of NMS, negatively affecting their quality of life. Among them, GI affection is the most frequent. Due to impaired ENS, constipation and delayed gastric emptying occur. Both conditions are associated with the occurrence of dysbiosis, and more specifically, SIBO. In the long term, this can also affect the motor situation of these patients by leading to disturbances in levodopa absorption.

The results of this study could play a potential role in the management of SIBO predisposing conditions, provide additional data to the current knowledge on the role of dysbiosis in PD patients, and open a way to important lines of research.

Assuming that the presented hypotheses are confirmed, and proven the use of probiotics as an add-on treatment to rifaximin in PD patients suffering from SIBO and motor fluctuations is effective in reducing the relapse rates, patients would benefit from its implementation by avoiding an unnecessary increase in the dose and frequency of levodopa and thus, the possibility of dyskinesias. It will also improve their quality of life, as NMS are as disabling as motor symptoms. In addition, this will also have an impact on the National Healthcare System, as levodopa doses will not need to be increased as frequently, which is also in their best economic interest.

16. ANNEXES

ANNEX I

Hoehn and Yahr Stages

Table 7. *Hoehn and Yahr stages.*

Stage	Hoehn and Yahr Scale
0	No signs of disease
1	Unilateral involvement
1,5	Unilateral involvement with axial affection
2	Bilateral involvement but no impairment of balance
2,5	Bilateral involvement with a balance impairment
3	Mild to moderate bilateral disease; balance instability; physically independent
4	Severe disability, but still able to walk or stand unassisted
5	Disabled in a wheel-chair or bed

ANNEX II

The Unified Parkinson's Disease Rating Scale – III (motor)

Table 8. *UPDRS-III(38)*

3a. Is the patient on medication for treating the symptoms of Parkinson's disease?	☐ **No** ☐ **Yes**
3b. If the patient is receiving medication for treating the symptoms of Parkinson's disease, mark the patient's clinical state using the following definitions:	☐ ON: On is the typical functional state when patients are receiving medication and have a good response. ☐ OFF: Off is the typical functional state when patients have a poor response in spite of taking medications
3c. Is the patient on levodopa?	☐ No ☐ Yes **III.c1.** If yes, minutes since last levodopa dose: _____
3.1. Speech **<u>Instructions to examiner:</u> Listen to the patient's free-flowing speech and engage in conversation if necessary. Suggested topics: ask about the patient's work, hobbies, exercise, or how he got to the doctor's office. Evaluate volume, modulation (prosody) and clarity, including slurring, palilalia (repetition of syllables), and tachyphemia (rapid speech, running syllables together).** **0: Normal: No speech problems.** **1: Slight: Loss of modulation, diction, or volume, but still all words easy to understand.** **2: Mild: Loss of modulation, diction, or volume, with a few words unclear, but the overall sentences easy to follow** **3: Moderate: Speech is difficult to understand to the point that some, but not most, sentences are poorly understood.** **4: Severe: Most speech is difficult to understand or unintelligible.**	SCORE:

3.2. Facial expression **<u>Instructions to examiner:</u> Observe the patient sitting at rest for 10 seconds, without talking and also while talking. Observe eye-blink frequency, masked facies or loss of facial expression, spontaneous smiling, and parting of lips.** **0: Normal: Normal facial expression.** **1: Slight: Minimal masked facies manifested only by decreased frequency of blinking.** **2: Mild: In addition to decreased eye-blink frequency, masked facies present in the lower face as well, namely fewer movements around the mouth, such as less spontaneous smiling, but lips not parted.** **3: Moderate: Masked facies with lips parted some of the time when the mouth is at rest.** **4: Severe: Masked facies with lips parted most of the time when the mouth is at rest.**	SCORE:
3.3.Rigidity **<u>Instructions to examiner:</u> Rigidity is judged on slow passive movement of major joints with the patient in a relaxed position and the examiner manipulating the limbs and neck. First, test without an activation maneuver. Test and rate neck and each limb separately. For arms, test the wrist and elbow joints simultaneously. For legs, test the hip and knee joints simultaneously. If no rigidity is detected, use an activation maneuver such as tapping fingers, fist opening/closing, or heel tapping in a limb not being tested. Explain to the patient to go as limp as possible as you test for rigidity.** **0: Normal: No rigidity.** **1: Slight: Rigidity only detected with activation maneuver.** **2: Mild: Rigidity detected without the activation maneuver, but full range of motion is easily achieved.** **3: Moderate: Rigidity detected without the activation maneuver; full range of motion is achieved with effort.** **4: Severe: Rigidity detected without the activation maneuver and full range of motion not achieved.**	SCORE:

3.4.Finger tapping	SCORE
<u>Instructions to examiner:</u> Each hand is tested separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to tap the index finger on the thumb 10 times as quickly AND as big as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts, and decrementing amplitude. **0: Normal: No problems.** **1: Slight: Any of the following:** **a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement;** **b) slight slowing;** **c) the amplitude decrements near the end of the 10 taps.** **2: Mild: Any of the following:** **a) 3 to 5 interruptions during tapping;** **b) mild slowing;** **c) the amplitude decrements midway in the 10-tap sequence.** **3: Moderate: Any of the following:** **a) more than 5 interruptions during tapping or at least one longer arrest (freeze) in ongoing movement;** **b) moderate slowing;** **c) the amplitude decrements starting after the 1st tap.** **4: Severe: Cannot or can only barely perform the task because of slowing, interruptions, or decrements.**	
3.5. Hand movements	SCORE
<u>Instructions to examiner:</u> Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to make a tight fist with the arm bent at the elbow so that the palm faces the examiner. Have the patient open the hand 10 times as fully AND as quickly as possible. If the patient fails to make a tight fist or to open the hand fully, remind him/ her to do so. Rate each side separately, evaluating speed, amplitude, hesitations, halts, and decrementing amplitude. **0: Normal: No problems.** **1: Slight: Any of the following:** **a) the regular rhythm is broken with one or two interruptions or hesitations of the movement;** **b) slight slowing;** **c) the amplitude decrements near the end of the task.**	

2: Mild: Any of the following: **a) 3 to 5 interruptions during the movements;** **b) mild slowing;** **c) the amplitude decrements midway in the task.** **3: Moderate: Any of the following:** **a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement;** **b) moderate slowing;** **c) the amplitude decrements starting after the 1st open-and-close sequence.** **4: Severe: Cannot or can only barely perform the task because of slowing, interruptions, or decrements.**	
3.6.Pronation-supination movements of hands **<u>Instructions to examiner:</u> Test each hand separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to extend the arm out in front of his/her body with the palms down, and then to turn the palm up and down alternately 10 times as fast and as fully as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts, and decrementing amplitude.** **0: Normal: No problems.** **1: Slight: Any of the following:** **a) the regular rhythm is broken with one or two interruptions or hesitations of the movement;** **b) slight slowing;** **c) the amplitude decrements near the end of the sequence.** **2: Mild: Any of the following:** **a) 3 to 5 interruptions during the movements;** **b) mild slowing;** **c) the amplitude decrements midway in the sequence.** **3: Moderate: Any of the following:** **a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement;** **b) moderate slowing;** **c) the amplitude decrements starting after the 1st supination-pronation sequence.** **4: Severe: Cannot or can only barely perform the task because of slowing, interruptions, or decrements.**	SCORE

3.7. Toe tapping **<u>Instructions to examiner:</u> Have the patient sit in a straight-backed chair with arms, both feet on the floor. Test each foot separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the heel on the ground in a comfortable position and then tap the toes 10 times as big and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts, and decrementing amplitude.** **0: Normal: No problems.** **1: Slight: Any of the following:** **a) the regular rhythm is broken with one or two interruptions or hesitations of the tapping movement;** **b) slight slowing;** **c) amplitude decrements near the end of the ten taps.** **2: Mild: Any of the following:** **a) 3 to 5 interruptions during the tapping movements;** **b) mild slowing;** **c) amplitude decrements midway in the task.** **3: Moderate: Any of the following:** **a) more than 5 interruptions during the tapping movements or at least one longer arrest (freeze) in ongoing movement;** **b) moderate slowing;** **c) amplitude decrements after the 1st tap.** **4: Severe: Cannot or can only barely perform the task because of slowing, interruptions or decrements.**	SCORE
3.8. Leg agility **<u>Instructions to examiner:</u> Have the patient sit in a straight-backed chair with arms. The patient should have both feet comfortably on the floor. Test each leg separately. Demonstrate the task, but do not continue to perform the task while the patient is being tested. Instruct the patient to place the foot on the ground in a comfortable position and then raise and stomp the foot on the ground 10 times as high and as fast as possible. Rate each side separately, evaluating speed, amplitude, hesitations, halts and decrementing amplitude.** **0: Normal: No problems.**	SCORE

1: Slight: Any of the following: **a) the regular rhythm is broken with one or two interruptions or hesitations of the movement;** **b) slight slowing;** **c) amplitude decrements near the end of the task.** **2: Mild: Any of the following:** **a) 3 to 5 interruptions during the movements;** **b) mild slowness;** **c) amplitude decrements midway in the task.** **3: Moderate: Any of the following:** **a) more than 5 interruptions during the movement or at least one longer arrest (freeze) in ongoing movement;** **b) moderate slowing in speed;** **c) amplitude decrements after the 1st tap.** **4: Severe: Cannot or can only**	
3.9. Arising from chair **<u>Instructions to examiner:</u> Have the patient sit in a straight-backed chair with arms, with both feet on the floor and sitting back in the chair (if the patient is not too short). Ask the patient to cross his/her arms across the chest and then to stand up. If the patient is not successful, repeat this attempt up to a maximum of two more times. If still unsuccessful, allow the patient to move forward in the chair to arise with arms folded across the chest. Allow only one attempt in this situation. If unsuccessful, allow the patient to push off using his/her hands on the arms of the chair. Allow a maximum of three trials of pushing off. If still not successful, assist the patient to arise. After the patient stands up, observe the posture for item 3.13.** **0: Normal: No problems. Able to arise quickly without hesitation.** **1: Slight: Arising is slower than normal; or may need more than one attempt; or may need to move forward in the chair to arise. No need to use the arms of the chair.** **2: Mild: Pushes self up from the arms of the chair without difficulty.** **3: Moderate: Needs to push off, but tends to fall back; or may have to try more than one time using the arms of the chair, but can get up without help.** **4: Severe: Unable to arise without help.**	SCORE

3.10. Gait **<u>Instructions to examiner:</u> Testing gait is best performed by having the patient walking away from and towards the examiner so that both right and left sides of the body can be easily observed simultaneously. The patient should walk at least 10 meters (30 feet), then turn around and return to the examiner. This item measures multiple behaviors: stride amplitude, stride speed, height of foot lift, heel strike during walking, turning, and arm swing, but not freezing. Assess also for "freezing of gait" (next item 3.11) while patient is walking. Observe posture for item 3.13.** **0: Normal: No problems.** **1: Slight: Independent walking with minor gait impairment.** **2: Mild: Independent walking but with substantial gait impairment.** **3: Moderate: Requires an assistance device for safe walking (walking stick, walker) but not a person.** **4: Severe: Cannot walk at all or only with another person's assistance.**	SCORE
3.11. Freezing of gait **<u>Instructions to examiner:</u> While assessing gait, also assess for the presence of any gait freezing episodes. Observe for start hesitation and stuttering movements especially when turning and reaching the end of the task. To the extent that safety permits, patients may NOT use sensory tricks during the assessment.** **0: Normal: No freezing.** **1: Slight: Freezes on starting, turning, or walking through doorway with a single halt during any of these events, but then continues smoothly without freezing during straight walking.** **2: Mild: Freezes on starting, turning, or walking through doorway with more than one halt during any of these activities, but continues smoothly without freezing during straight walking.** **3: Moderate: Freezes once during straight walking.** **4: Severe: Freezes multiple times during straight walking.**	SCORE

3.12. Postural stability **<u>Instructions to examiner:</u> The test examines the response to sudden body displacement produced by a quick, forceful pull on the shoulders while the patient is standing erect with eyes open and feet comfortably apart and parallel to each other. Test retropulsion. Stand behind the patient and instruct the patient on what is about to happen. Explain that s/he is allowed to take a step backwards to avoid falling. There should be a solid wall behind the examiner, at least 1-2 meters away to allow for the observation of the number of retropulsive steps. The first pull is an instructional demonstration and is purposely milder and not rated. The second time the shoulders are pulled briskly and forcefully towards the examiner with enough force to displace the centre of gravity so that patient MUST take a step backwards. The examiner needs to be ready to catch the patient, but must stand sufficiently back so as to allow enough room for the patient to take several steps to recover independently. Do not allow the patient to flex the body abnormally forward in anticipation of the pull. Observe for the number of steps backwards or falling. Up to and including two steps for recovery is considered normal, so abnormal ratings begin with three steps. If the patient fails to understand the test, the examiner can repeat the test so that the rating is based on an assessment that the examiner feels reflects the patient's limitations rather than misunderstanding or lack of preparedness. Observe standing posture for item 3.13.** **0: Normal: No problems. Recovers with one or two steps.** **1: Slight: 3-5 steps, but subject recovers unaided.** **2: Mild: More than 5 steps, but subject recovers unaided.** **3: Moderate: Stands safely, but with absence of postural response; falls if not caught by examiner.** **4: Severe: Very unstable, tends to lose balance spontaneously or with just a gentle pull on the shoulders.**	SCORE
3.13. Posture **<u>Instructions to examiner:</u> Posture is assessed with the patient standing erect after arising from a chair, during walking, and while being tested for postural reflexes. If you notice poor posture, tell the patient to stand up straight and see if the posture improves (see option 2 below). Rate the worst posture seen in these three observation points. Observe for flexion and side-to-side leaning.** **0: Normal: No problems.** **1: Slight: Not quite erect, but posture could be normal for older person.**	SCORE

2: Mild: Definite flexion, scoliosis or leaning to one side, but patient can correct posture to normal posture when asked to do so. **3: Moderate: Stooped posture, scoliosis or leaning to one side that cannot be corrected volitionally to a normal posture by the patient.** **4: Severe: Flexion, scoliosis or leaning with extreme abnormality of posture.**	
3.14. Global spontaneity of movement (body bradykinesia) **<u>Instructions to examiner:</u> This global rating combines all observations on slowness, hesitancy, and small amplitude and poverty of movement in general, including a reduction of gesturing and of crossing the legs. This assessment is based on the examiner's global impression after observing for spontaneous gestures while sitting, and the nature of arising and walking.** **0: Normal: No problems.** **1: Slight: Slight global slowness and poverty of spontaneous movements.** **2: Mild: Mild global slowness and poverty of spontaneous movements.** **3: Moderate: Moderate global slowness and poverty of spontaneous movements.** **4: Severe: Severe global slowness and poverty of spontaneous movements.**	SCORE
3.16. Postural tremor of the hands **<u>Instructions to examiner:</u> All tremor, including re-emergent rest tremor, that is present in this posture is to be included in this rating. Rate each hand separately. Rate the highest amplitude seen. Instruct the patient to stretch the arms out in front of the body with palms down. The wrist should be straight and the fingers comfortably separated so that they do not touch each other. Observe this posture for 10 seconds.** **0: Normal: No tremor.** **1: Slight: Tremor is present but less than 1 cm in amplitude.** **2: Mild: Tremor is at least 1 but less than 3 cm in amplitude.** **3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude.** **4: Severe: Tremor is at least 10 cm in amplitude**	SCORE

3.16. Kinetic tremor of the hands **<u>Instructions to examiner:</u> This is tested by the finger-to-nose maneuver. With the arm starting from the outstretched position, have the patient perform at least three finger-to-nose maneuvers with each hand reaching as far as possible to touch the examiner's finger. The finger-to-nose maneuver should be performed slowly enough not to hide any tremor that could occur with very fast arm movements. Repeat with the other hand, rating each hand separately. The tremor can be present throughout the movement or as the tremor reaches either target (nose or finger). Rate the highest amplitude seen.** **0: Normal: No tremor.** **1: Slight: Tremor is present but less than 1 cm in amplitude.** **2: Mild: Tremor is at least 1 but less than 3 cm in amplitude.** **3: Moderate: Tremor is at least 3 but less than 10 cm in amplitude.** **4: Severe: Tremor is at least 10 cm in amplitude.**	SCORE
3.17. Rest tremor amplitude **<u>Instructions to examiner:</u> This and the next item have been placed purposefully at the end of the examination to allow the rater to gather observations on rest tremor that may appear at any time during the exam, including when quietly sitting, during walking, and during activities when some body parts are moving but others are at rest. Score the maximum amplitude that is seen at any time as the final score.** **Rate only the amplitude and not the persistence or the intermittency of the tremor.** **As part of this rating, the patient should sit quietly in a chair with the hands placed on the arms of the chair (not in the lap) and the feet comfortably supported on the floor for 10 seconds with no other directives. Rest tremor is assessed separately for all four limbs and also for the lip/jaw. Rate only the maximum amplitude that is seen at any time as the final rating.** ***Extremity ratings*** **0: Normal: No tremor.** **1: Slight: < 1 cm in maximal amplitude.** **2: Mild: ≥ 1 cm but < 3 cm in maximal amplitude.** **3: Moderate: ≥ 3 cm but < 10 cm in maximal amplitude.** **4: Severe: ≥ 10 cm in maximal amplitude.**	SCORE

Lip/Jaw ratings **0: Normal: No tremor.** **1: Slight: < 1 cm in maximal amplitude.** **2: Mild: ≥ 1 cm but < 2 cm in maximal amplitude.** **3: Moderate: ≥ 2 cm but < 3 cm in maximal amplitude.** **4: Severe: ≥ 3 cm in maximal amplitude.**	
3.18. Constancy of rest tremor **Instructions to examiner: This item receives one rating for all rest tremor and focuses on the constancy of rest tremor during the examination period when different body parts are variously at rest. It is rated purposefully at the end of the examination so that several minutes of information can be coalesced into the rating.** **0: Normal: No tremor.** **1: Slight: Tremor at rest is present ≤ 25% of the entire examination period.** **2: Mild: Tremor at rest is present 26-50% of the entire examination period.** **3: Moderate: Tremor at rest is present 51-75% of the entire examination period.** **4: Severe: Tremor at rest is present > 75% of the entire examination period.**	SCORE

ANNEX III.

The Unified Dyskinesia Rating Scale

***Table 9**. UDysRS(39)*

Part 1.A. ON-Dyskinesia	
Time spent with *ON*-Dyskinesia	
Instructions to patient (and caregiver): **Over the past week, how many hours do you usually sleep on a daily basis, including night-time sleep and daytime napping? Alright, if you sleep ___ hrs, you are awake ____ hrs. Out of those awake hours, how many hours in total are your medications working to control your Parkinson's disease (hours on ____)? During the hours that your medications are working, do you have jerking or twisting movements? Do not count the times when you have tremor, which is a regular back and forth shaking or times when you have painful cramps or spasms when you have not taken medication or when the medications for Parkinson's disease are not working. I will ask about those later. Concentrate only on these types of jerking or twisting movements that occur when your Parkinson's medicine is working.**	1.Total Hours *ON*: ___ 2.Total Hours of *ON*-Dyskinesia: ___ % *ON*-Dyskinesia = ((2/1)*100): ___ 0: Normal: No dyskinesia 1: Slight: ≤ 25% of *ON*- time 2: Mild: 26-50% of *ON*- time 3: Moderate: 51-75% of *ON*- time 4: Severe: >75% of *ON*- time
Part 1.B. Patient Dyskinesia Questionnaire	
2. Speech: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause problems with your speech? Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problems.** **1: Slight: Dyskinesias were present, but they did not interfere with my speech.** **2: Mild: Dyskinesias caused a few problems with my speech and people asked me to repeat myself occasionally.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid talking when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, most or all of my speech could not be understood.**	SCORE

3. Chewing and swallowing: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause problems swallowing pills or eating meals? Did you need your pills cut or crushed or your meals to be made soft, chopped or blended to avoid choking? Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problems.** **1: Slight: Dyskinesias were present, but they did not interfere with my chewing or swallowing.** **2: Mild: Dyskinesias caused a few problems with chewing and swallowing and it took me longer to chew or swallow because of on-dyskinesias.** **3: Moderate. Dyskinesias caused enough problems that I tried to avoid chewing and swallowing when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, I was unable to chew or swallow at all.**	SCORE
4. Eating tasks: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause troubles handling your food and using eating utensils? For example, did you have trouble handling finger foods or using forks, knifes, spoons, chopsticks? Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problems.** **1: Slight: Dyskinesias were present, but they did not interfere with my eating.** **2: Mild: Dyskinesias caused a few problems with eating and it took me longer to eat because of on-dyskinesias.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid eating when I had on dyskinesias.** **4: Severe: When I had dyskinesias, I needed help for most or all eating tasks.**	SCORE

5. Dressing: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause problems with your dressing? For example, did you need help with buttoning, using zippers, putting on or taking off your clothes or jewelry? Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problems.** **1: Slight: Dyskinesias were present but they did not interfere with dressing tasks.** **2: Mild: Dyskinesias caused a few problems with dressing and it took me longer to get dressed because of on-dyskinesias.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid getting dressed when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, I needed help for most or all dressing tasks.**	SCORE
6. Hygiene: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause problems with your personal hygiene? For example, did you need help with washing, bathing, shaving, brushing teeth, or combing your hair? Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problems.** **1: Slight: Dyskinesias were present but they did not interfere with hygiene tasks.** **2: Mild: Dyskinesias caused a few problems with hygiene tasks and it took me longer to do these activities because of on-dyskinesias.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid doing hygiene tasks when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, I needed help for most or all of my hygiene tasks.**	SCORE

7. Handwriting: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause trouble with your handwriting. Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problems.** **1: Slight: Dyskinesias were present, but they did not interfere with my handwriting.** **2: Mild: Dyskinesias caused a few problems with writing and it took me longer to write because of on-dyskinesias.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid writing when I had on dyskinesias.** **4: Severe: When I had dyskinesias, most or all words could not be read.**	SCORE
8. Doing hobbies and other activities: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on dyskinesias usually cause trouble doing your hobbies or other things that you like to do? Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problems.** **1: Slight: Dyskinesias were present but they did not interfere with these activities.** **2: Mild: Dyskinesias caused a few problems with these activities and it took me longer to do them because of on-dyskinesias.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid doing hobbies or other activities when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, I was unable to do most or all of these activities.**	SCORE
9. Walking and balance: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause problems with balance and walking. Consider only effects of dyskinesias, not problems caused by Parkinson's disease.**	SCORE

0: Normal: Not at all, no problems. **1: Slight: Dyskinesias were present but they did not interfere with walking or balance.** **2: Mild: Dyskinesias caused a few problems with walking. It took me longer to walk because of on-dyskinesias and I occasionally bumped into things.** **3: Moderate: Dyskinesias caused enough problems that I usually used a walking aid (cane, walker) to walk safely without falling. However, I did not usually need the support of another person. I tried to avoid walking when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, I could not walk safely without falling.**	
10. Public and social settings: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on-dyskinesias usually cause problems when you were dealing with other people or in public? Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problem.** **1: Slight: Dyskinesias were present but they did not interfere with these activities.** **2: Mild: Dyskinesias caused a few problems and I was self-conscious in public but I did not avoid social situations.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid some social situations when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, I could not be with people, even friends or family.**	SCORE
11. Exciting or emotional settings: **Over the past week, when your Parkinson's disease medications were working, did jerking or twisting movements called on dyskinesias usually cause problems during emotional conversations, exciting movies, or other highly stimulating situations. Consider only effects of dyskinesias, not problems caused by Parkinson's disease.** **0: Normal: Not at all, no problem.**	SCORE

1: Slight: Dyskinesias were present, but they did not interfere with these activities. **2: Mild: Dyskinesias caused few problems.** **3: Moderate: Dyskinesias caused enough problems that I tried to avoid some exciting situations when I had on-dyskinesias.** **4: Severe: When I had dyskinesias, I could not stay in exciting situations.**	
Part 2.A. OFF-Dystonia	
Time spent with *OFF*-Dystonia	
Over the past week, on a typical day, think about the number of hours of the day when you are stiff and slow, whether this is before you take morning medications, perhaps late in the evening, or during the day when the good effects of medication have worn out. Within these "OFF" times, how many hours or minutes do you have spasms or cramps that we call OFF-dystonia? **0 = Never** **1 = Less than 30 minutes a day** **2 = Less than 60 minutes a day.** **3 = Less than 2 hours a day.** **4 = Greater than 2 hours a day.**	SCORE
Part 2.B. Patient Dyskinesia Questionnaire	
13. Effects or spasms or cramps called off-dystonia separate from pain on activities. **During the past week, separate from pain, have spasms or cramps called off dystonia occurred?** **0: Normal: Not at all.** **1: Slight: Off-dystonia occurred but it did not interfere with my daily activities.** **2: Mild: Off-dystonia caused a few problems and it took me longer to do activities because of off-dystonia.** **3: Moderate: Off-dystonia caused enough problems that I avoided doing these activities when I had off-dystonia.** **4: Severe: When off-dystonia occurred, I could not do many activities.**	SCORE

14. Effects of pain from off-dystonia on daily activities: On average during this past week, if spasms or cramps called off-dystonia occurred, did pain limit your activities? **0: Normal: Not at all, no pain from off-dystonia.** **1: Slight: I had pain from off-dystonia, but it did not limit my activities** **2: Mild: Pain from off-dystonia caused a few problems and it took me longer to do activities because of pain from off-dystonia.** **3: Moderate: Pain from off-dystonia caused enough problems that I avoided doing these activities when I had pain from off-dystonia.** **4: Severe: Because of pain from dystonia, I could not do many activities.**	SCORE
15. Dystonia pain: On average during the past week, how severe was the pain from the spasms or cramps of off-dystonia? **0: Normal: Not painful** **1: Slight: Mild ache or discomfort.** **2: Mild: Moderate ache and discomfort.** **3: Moderate: Severe discomfort.** **4: Severe: Excruciating pain.**	SCORE

Part 3. Patient Disability and Impairment **In this section, you will observe the patient or observe a videotape of the patient during four activities of daily living.**		
Communication	Face Neck R.arm/shoulder L.arm/shoulder Trunk R.leg/hip L.leg/hip	0=No dyskinesia 1=Dyskinesia present but does not impair communication 2=Dyskinesia impairs communication but patient is fully understandable 3=Dyskinesia interferes with communication such that parts of communication cannot be understood but overall content is understandable 4=Dyskinesia interferes with comprehension of overall communication
Drinking	Face Neck R.arm/shoulder L.arm/shoulder Trunk R.leg/hip L.leg/hip	0=No dyskinesia observed 1=Dyskinesia present but it does not affect performance of the task 2=Dyskinesia affects the smooth performance but causes no splashing or spilling 3=Dyskinesia affects performance such that patient spills a few drops of water 4=Dyskinesia affects performance such that patient spills more than a few drops or dyskinesia cause coughing or choking.

Dressing	Face	0=No dyskinesia observed 1=Dyskinesia present but does not interfere with or slow dressing 2=Dyskinesia affects smooth performance of task but the performance is at most minimally slowed 3=Dyskinesia interferes and slows performance but it is completed within 60 seconds 4=Dyskinesia precludes completing the task within 60 seconds
	Neck	
	R.arm/shoulder	
	L.arm/shoulder	
	Trunk	
	R.leg/hip	
	L.leg/hip	
Ambulation	Face	0=No dyskinesia observed 1=Mild dyskinesia present but does not alter normal synchrony or cadence 2=Dyskinesia is present which alters the normal cadence of rising, sitting or walking but does not slow overall performance. 3=Dyskinesia is present which disrupts or distorts arising, sitting or walking. Performance is slowed. Patient is able to rise and walk without imminent danger of falling. 4=Dyskinesia prohibits walking safely without assistance
	Neck	
	R.arm/shoulder	
	L.arm/shoulder	
	Trunk	
	R.leg/hip	
	L.leg/hip	

ANNEX IV

CATALAN VERSION

FULL INFORMATIU PEL PACIENT

Introducció:

Ens dirigim a vostè per informar-lo/la sobre la realització d'un estudi al qual ens agradaria que participés. Aquest estudi ha estat aprovat pel Comitè Ètic de Recerca Clínica del seu hospital de referència amb la legislació vigent, Llei 14/2007, del 3 de juliol, de recerca biomèdica amb procediments invasius.

El nostre propòsit és que vostè rebi informació precisa y veraç per tal que pugui valorar i decidir si vol participar o no en aquest estudi. Llegiu aquest full informatiu amb atenció i l'equip s'encarregarà de clarificar els possibles dubtes que puguin sorgir. A més, podreu consultar a les persones que considereu pertinents.

Títol de l'assaig clinic: Probiotics as an add-on treatment for Parkinson's disease patients suffering from motor fluctuations and dysbiosis

Participació voluntària:

En primer lloc, ens agradaria informar-lo que la seva participació en aquest estudi és voluntària. Això significa que si no vol fer-ho, o que si en haver començat, canvia d'opinió, podrà retirar el seu consentiment en qualsevol moment. La negativa no canviarà la relació amb el seu metge ni provocarà cap extorsió pel que fa al tractament.

Descripció de l'estudi:

La malaltia del Parkinson és la segona malaltia neurodegenerativa més freqüent en el nostre medi. Tot i que acostuma a diagnosticar-se a partir dels símptomes motors que els pacients presenten (tremolor, bradicinèsia, i rigidesa), hi ha tot un espectre de símptomes no motors que també afecten la qualitat de vida del pacient. Entre aquests, els símptomes gastrointestinals com l'estrenyiment i el retard del buidament gàstric són molt freqüents, i apareixen fins i tot molt abans que els símptomes motors per una alteració del sistema nerviós gastrointestinal i per la disbiosis present.

Amb el temps, aquestes entitats tenen repercussions en el pacient, provocant un sobrecreixement bacterià en l'àmbit intestinal (SIBO) que impedeix la correcta absorció del levodopa, el fàrmac principal que s'utilitza en el tractament. I aquestes alteracions serien un dels motius principals de l'aparició de fluctuacions motores en les fases inicials de la malaltia. Hi ha evidència científica que demostra que tractant aquest sobrecreixement, les fluctuacions milloren. Però com que els factors predisposants encara són presents, gairebé la meitat dels pacients acaben recidivant.

Amb el nostre estudi, plantegem la possibilitat d'afegir probiòtics en el posttractament del SIBO amb l'objectiu de regular aquests factors predisposants i la flora microbiana de l'aparell digestiu, de manera que la fisiologia normal d'aquest es recuperi i els percentatges de recidiva disminueixin. Creiem que, d'aquesta manera, es podran regular millor les fluctuacions motores dels pacients, millorant llur qualitat de vida.

Beneficis i riscos:

Abans de participar en l'estudi, avalueu els avantatges i els inconvenients i prengui la seva decisió en funció d'aquests. El SIBO no és una entitat que es diagnostiqui per rutina, tot i haver-se demostrat com una de les causes de l'aparició de fluctuacions motores. Tractar aquesta entitat ja planteja molts beneficis, i si a més a més afegim probiòtics, aquests beneficis es podrien allargar en el temps. Addicionalment, els probiòtics han demostrat tenir pocs efectes adversos. Pensem que, afegint aquest suplement, el pacient s'exposa a més punts positius que negatius.

Els possibles inconvenients de la participació són:

- Qualsevol efecte advers per l'ús dels probiòtics.
- Qualsevol efecte advers per l'ús de l'antibiòtic (rifaximina): Mareig, cefalees, dolor abdominal, pirèxia, reacció al·lèrgica.
- Risc de l'aparició de discinèsies per la millora de la biodisponibilitat de levodopa, tot i que això es podria millorar reajustant les dosis.
- Efectes adversos relacionats amb la presa mostres sanguínies: sagnat excessiu, hematoma, infecció, dolor, sensació de mareig.

Compensació econòmica:

La participació en aquest estudi serà totalment voluntària, de manera que els participants no rebran cap compensació econòmica.

Confidencialitat:

Sol·licitem el seu permís per participar en el següent estudi i utilitzar les seves dades. El tractament, la comunicació i la cessió de les dades de caràcter personal de tots els individus participants s'ajustarà d'acord amb la Llei Orgànica 3/2018, del 5 de desembre, de protecció de dades personals i garantia dels drets digitals. Segons el que s'estableix a la legislació esmentada, vostè pot exercir els drets d'accés, modificació, oposició i cancel·lació de dades; i s'haurà de dirigir al responsable de l'estudi per deixar constància de la seva decisió.

Addicionalment, heu de saber que en cap moment figuraran les seves dades personals en l'estudi, i d'acord amb el Reglament General de Protecció de Dades (Reglament 2016/679 del Parlament Europeu i del Consell del 27 d'abril de 2016 i el respectiu desenvolupament a Espanya), les sotmetrem a un procés d'anonimització de manera que ningú extern al projecte pugui relacionar-lo amb el mateix. L'hospital es responsabilitza del maneig d'aquesta informació i es compromet a complir aquesta normativa en vigor.

Revocació del consentiment:

Heu de saber que, sempre que ho desitgeu, podeu interrompre la vostra participació en el projecte sense necessitat de justificar-se.

Contacte:

Si té dubtes, pot posar-se en contacte amb el nostre equip d'investigació a través d'alguna de les següents vies:

Telèfon: _____

Correu electrònic: _____

Moltes gràcies.

SPANISH VERSION

HOJA INFORMATIVA PARA EL PACIENTE

Introducción:

Nos dirigimos a usted para informarlo/la sobre la realización de un estudio en el cual nos gustaría que participara. Este estudio ha sido aprobado por el Comité Ético de Recerca Clínica de su hospital de referencia con la legislación vigente, Ley 14/2007, del 3 de julio, de recerca biomédica con procedimientos invasivos.

Nuestro propósito es que usted reciba información precisa y veraz con el objetivo de que pueda valorar y decidir si quiere participar o no en este estudio. Leed esta hoja informativa con atención y el equipo se encargar de clarificar las posibles dudas que le puedan surgir. Además, podréis consultar a las personas que consideréis pertinentes.

Título del ensayo clínico: Probiotics as an add-on treatment for Parkinson's disease patients suffering from motor fluctuations and dysbiosis

Participación voluntaria:

En primer lugar, nos gustaría informarlo/la de su participación en este estudio es voluntaria. Esto significa que, si no quiere hacerlo, o que si al empezar, cambia de opinión, podrá retirar su consentimiento en cualquier momento. La negativa no cambiará la relación con su médico ni provocará ninguna extorsión en lo que al tratamiento se refiere.

Descripción del estudio:

La enfermedad del Parkinson es la segunda enfermedad neurodegenerativa más frecuente en nuestro medio. Aunque acostumbra a diagnosticarse a partir de los síntomas motores que los pacientes presentan (temblor, bradicinesia, y rigidez), hay todo un espectro de síntomas no motores que también afectan a la calidad de vida del paciente. Entre estos, los síntomas gastrointestinales como el estreñimiento y el retraso en el vaciamiento gástrico son muy frecuentes, y aparecen incluso mucho antes que los síntomas motores por una alteración en el sistema nervioso gastrointestinal y por la disbiosis presente.

Con el tiempo, estas entidades tienen repercusiones en el paciente, provocando un sobrecrecimiento bacteriano en el ámbito intestinal (SIBO) que impide la correcta absorción de la levodopa, el fármaco principal que se usa en el tratamiento. I estas alteraciones serian uno de los motivos principales por las cuales aparecen fluctuaciones motoras en las fases iniciales de la enfermedad. Hay evidencia científica que demuestra que, tratando este sobrecrecimiento, las fluctuaciones mejoran. Sin embargo, como los factores predisponentes todavía están presentes, casi la mitad de los pacientes acaban recidivando.

Con nuestro estudio planteamos la posibilidad de añadir probióticos al postratamiento del SIBO con el objetivo de regular estos factores predisponentes y la flora microbiana del aparato digestivo, de modo que la fisiología normal de este se recupere y los porcentajes de recidiva disminuyan. Creemos que, así, se podrán regular mejor las fluctuaciones motoras en los pacientes, mejorando su calidad de vida.

Beneficios y riesgos:

Antes de participar en el estudio, avalúe las ventajas y los inconvenientes y tome su decisión en función de estas. El SIBO no es una entidad que diagnostique por rutina, aunque haya demostrado ser una de las causas de la aparición de fluctuaciones motoras. Tratar esta entidad ya plantea muchos beneficios, y si además añadimos probióticos, estos beneficios se podrían alargar en el tiempo. Adicionalmente, los probióticos han demostrado tener pocos efectos adversos. Pensamos que, al añadir este suplemento, el paciente se expone a más puntos positivos que negativos.

Los posibles inconvenientes en su participación podrían ser:

- Cualquier efecto adverso relacionado con el uso de los probióticos.
- Cualquier efecto adverso relacionado con el antibiótico (rifaximina): Mareo, cefalea, dolor abdominal, pirexia, reacción alérgica.
- Riesgo de aparición de discinesias por la mejora de la biodisponibilidad de la levodopa, aunque esto podría revertirse reajustando la dosis.
- Efectos adversos relacionados con la toma de muestras sanguíneas: sangrado excesivo, hematoma, infección, dolor, sensación de mareo.

Compensación económica:

La participación en este estudio será totalmente voluntaria, de modo que los participantes no recibirán ninguna compensación económica.

Confidencialidad:

Solicitamos su permiso para participar en el siguiente estudio y poder hacer uso de sus datos. El tratamiento, la comunicación y la cesión de los datos de carácter personal de todos los individuos participantes se ajustará de acuerdo con la Ley Orgánica 3/2018, del 5 de diciembre, de protección de datos personales y garantía de los derechos digitales. Según lo que se establece en la legislación mencionada, usted puede ejercer los derechos de acceso, modificación, oposición y cancelación de datos; y deberá dirigirse al responsable del estudio para dejar constancia de su decisión.

Adicionalmente, debe saber que en ningún momento figurarán sus datos personales en el estudio, y de acuerdo con el Reglamento General de Protección de Datos (Reglamento 2016/679 del Parlamento Europeo y del Consejo del 27 de abril de 2016 y el respectivo desarrollo en España), los someteremos a un proceso de anonimización de modo que nadie externo al proyecto pueda relacionarlo con el mismo. El hospital se responsabiliza de la gestión de esta información y se compromete a cumplir esta normativa en vigor.

Revocación del consentimiento:

Debe saber que, siempre que así lo desee, puede interrumpir su participación en el proyecto sin necesidad de justificarse.

Contacto:

Si tiene dudas, puede ponerse en contacto con nuestro equipo de investigación a través de una de las siguientes vías:

Teléfono: _____

Correo electrónico: _____

Muchas gracias.

ANNEX V

CATALAN VERSION

CONSENTIMENT INFORMAT

Títol de l'assaig clinic: Probiotics as an add-on treatment for Parkinson's disease patients suffering from motor fluctuations and dysbiosis

Hospital: ……

Investigador/a principal: ……

Jo, (nom i cognoms del pacient): ……

- ☐ He llegit el full informatiu
- ☐ He rebut informació suficient sobre l'estudi
- ☐ He pogut consultar els meus dubtes amb els professionals

He parlat amb (nom de l'investigador/a): ……

- ☐ Entenc que la meva participació és voluntària.
- ☐ Entenc que puc retirar-me de l'estudi:
  - Quan vulgui
  - Sense haver de justificar-me
  - Sense que tingui repercussions en l'atenció mèdica que rebré

Aprovo la meva conformitat per participar en aquest estudi i dono el meu consentiment per accedir i utilitzar a les dades en les condicions detallades en el full informatiu.

Nom i cognoms del pacient: Signatura del pacient: Data: ..../..../.....	Nom i cognoms de l'investigador/a: Signatura de l'investigador/a: Data: ..../..../.....

SPANISH VERSION

CONSENTIMIENTO INFORMADO

Título del ensayo clínico: Probiotics as an add-on treatment for Parkinson's disease patients suffering from motor fluctuations and dysbiosis

Hospital: ……

Investigador/a principal: ……

Yo, (nombre y apellidos del paciente): ……

- ☐ He leído la hoja informativa
- ☐ He recibido información suficiente sobre el estudio
- ☐ He podido consultar mis dudas con los profesionales

He hablado con (nombre del investigador/a): ……

- ☐ Entiendo que mi participación es voluntaria.
- ☐ Entiendo que puedo retirarme del estudio:
  - Cuando quiera
  - Sin tener que justificarme
  - Sin que tenga repercusiones en la atención médica que recibiré

Apruebo mi conformidad para participar en este estudio y doy mi consentimiento para acceder y usar los datos en las condiciones detalladas en la hoja informativa.

Nombre y apellidos del paciente: Signatura del paciente: Fecha: …/…/.....	Nombre y apellidos del paciente: Signatura del investigador/a: Fecha: …/…/.....

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