Low pyridoxine levels and its association with the onset of gastrointestinal intolerance to oral levodopa in patients with Parkinson's disease

A cohort study

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A tots els sanitaris que segueixen lluitant contra la COVID-19.

Index

1. ABSTRACT

TITLE
Low pyridoxine levels and its association with the onset of gastrointestinal intolerance to oral levodopa in patients with Parkinson's disease.

BACKGROUND
Parkinson's disease (PD) is the second most common neurodegenerative disease consisting of the presence of motor symptoms, bradykinesia plus stiffness, tremor or instability and other non-motor symptoms. PD has a high prevalence. The etiology of the disease is unknown in most cases although risk factors related to its occurrence have been identified. Its diagnosis is based on the clinic. There is no curative treatment but symptomatic treatments. Several pharmacological groups can be considered in patients with PD, but still after 50 years of its onset the choice for most patients is L-DOPA, the most effective drug to treat the symptoms of the disease. The progression of the disease over time implies the need to increase doses of this drug or changes in the form of administration. A high percentage of patients taking this drug have adverse reactions, most of them motor but a considerable percentage also present adverse digestive reactions. In the metabolic pathway of this drug participate some B- complex vitamins such as pyridoxine, cobalamin, folic acid, and other biomolecules such as homocysteine.

OBJECTIVE
The aim of this study is to assess whether low pyridoxine levels influence the onset of gastrointestinal intolerance to L-DOPA in patients with Parkinson’s disease.

DESIGN
The study is designed as a multicenter observational prospective cohort study in reference hospitals of Catalonia.

PARTICIPANTS
All patients with Parkinson's disease who start oral L-DOPA treatment (n=280) and meet the selection criteria will participate in this study.

METHODS
Pyridoxine and covariate values (B12, folic acid and homocysteine) will be obtained from blood tests. To assess gastrointestinal intolerance patients will respond a survey of gastrointestinal tolerance to L-DOPA within three months of starting treatment. Subsequently we will carry out a statistical analysis to compare which percentage of cases appears in relation to low levels of pyridoxine compared to the group that does not have them low.

KEY WORDS
Parkinson's disease, L-DOPA, gastrointestinal intolerance, pyridoxine

2. ABBREVIATIONS

AADC inhibitor	Inhibitors of peripheral amino acid decarboxylase
AEMPS	Agencia Española del Medicamento y Productos Sanitarios
ATC	Anatomical, Therapeutic, Chemical classification system
AUC	Area under the plasma concentration-time curve
BBB	Blood-brain barrier
CEIC	Comitè d'Ètica de Investigació Clínica
CFS	Cerebrospinal fluid
CI	Confidence interval
CNS	Central Nervous System
COMT- I	Catechol-O-methyltransferase inhibitor
CV	Coefficient of Variation
DA	Dopamine agonists
GERD	Gastroesophageal Reflux Disease
GIT Scales	Gastrointestinal Tolerance Scales
ICS	Institut Català de la Salut
IMAO-B	Monoamine oxidase-B inhibitors
LB	Lewy Bodies
L-DOPA	Levodopa
LN	Lewy Neurites
LP	Lewy pathology
MDS-PD	Movement Disorders Society
MSA-P	Multisystem atrophy
PD	Parkinson's Disease
PLP	5-phosphate pyridoxal coenzyme
PSP	Progressive supranuclear palsy
RF	Risk factor
RV	Reference Values
SN	Substantia nigra
TE	Total Error
Vitamin B12	Cobalamin
Vitamin B6	Pyridoxine
Vitamin B9	Folic acid

3. INTRODUCTION

3.1. Parkinson's Disease

3.1.1. Definition and epidemiology

Parkinson's disease (PD) is a neurodegenerative process that develops over time because of a dopamine deficit in the striatum of the brain and is characterized by the presence of multisystemic symptomatology, highlighting the rigid-akinetic syndrome with rest tremor. (1)

PD is the second degenerative neurological disease in frequency after Alzheimer's disease in people over the age of 65 and one of the most common movement disorders. (2) At the epidemiological level Parkinson's disease varies according to the characteristics of the studied population and the methods used. It affects all ethnicities and both sexes.

Incidence ranges from 8 to 19 /100,000 people-year in the general population. Depending on the age of presentation these values may vary, being lower in people under 40 years and increases considerably as the age increases from 50 cases /100,000 inhabitants-year at 65 years, up to 400 cases / 100.00 inhabitants-year at 85 years. (3) Regarding the difference between sexes we see that the incidence of PD is higher and statistically significant in men between the ages of 60 to 69 years (58.22 cases /100,000 inhabitants-year in men by 30.32 cases/ 10 0.000 inhabitants-year in the women) and also between the ages comprised of 70 to 79 years (162,58 cases/100.000 inhabitants-year in men by 93.32 cases/100.000 inhabitant-year in women). In ages above this the difference becomes non-significant. (4)

Table 1 - Meta-regression for gender differences in PD incidence between males and females

Age group	Cochrane Q subgroup analysis; pooled incidence rate (95% CI)		P value
	Female	Male	
40-49	3.26 (1.75-6.05)	3.57 (2.37-5.37)	0.7259
50-59	8.43 (5.74-12.40)	14.67 (8.74-24.60)	0.0893
60-69	30.32 (23.16-39.70)	58.22 (43.59-77.75)	0.0012*
70-79	93.32 (63.30-137.59)	162.58 (122.86-215.16)	0.023*
80+	103.48 (44.69-239.64)	258.47 (146.46-456.16)	0.0642
* Denotes significant value			

Data on the **prevalence of** PD are of interest because they can provide us with information useful to identify risk factors and understand better the natural history of the disease. The increase in life expectancy and demographic changes that are happening in many populations results in a progressive increase in people entering old age, therefore, an increased prevalence of neurodegenerative diseases.

According to studies this disease affects in a variable way ranging from 31 to 328 cases per 100,000 inhabitants in the general population.(3) If we take into account the

data exposed in the incidence section, we can also understand that if we looked at the prevalence by age group, prevalence would increase as the age increased, giving a prevalence above 1087 cases / 100,000 people at the age between 70-79 years and 1903 cases / 100,000 inhabitants in people over 80 years. Instead, the prevalence in patients between the ages of 40-49 is only 41 cases/100,000 inhabitants. (5) It is exceptional at ages below 30 (juvenile PD) and rare between the ages of 30 and 40 (early PD). (1)

3.1.2. Etiology and pathophysiology

Nowadays the etiopathogenesis of PD remains unknown, being the most frequent the idiopathic variant, as a result of the interaction between natural factors (aging), genetics and environmental factors.(1) Although several environmental factors have been found that demonstrate a significant relationship of association with the PD, many of these may present an inverse causality, they can present confusion, information biases, conflicts among others.(6)

Table 2 - Akinetic-rigid syndrome etiology (1)

Akinetic-rigid syndrome etiology	
Primary	**Secondary**
- Idiopathic PD - Genetically determined dominant o recessive PD - Other degenerative diseases: ▪ Multisystem atrophy (MSA-P) ▪ Progressive supranuclear palsy (PSP) ▪ Corticobasal degeneration ▪ Etc.	- Postencephalitic - Iatrogenic (pharmacological) - Exogenous toxins - Lacunar state (vascular parkinsonism) - Metabolic - Chronic post-traumatic encephalopathy - Etc.

Described risk factors related to the disease include environmental toxins, medicines, pesticides, brain microtrauma, focal cerebrovascular damage and genomic defects. (7) Despite all, age remains the most important RF. As age progresses there is a greater risk of presenting the disease as we have described in epidemiology section. Males are also considered a risk factor for PD as they show a discreet but significant difference from females (*Table 1)*. (3) Although several disease-related loci make up the genetically determined forms of PD, the reality is that only a small proportion of cases are defined by these findings. In this sense, we can say that the common hereditary components of the disease have not yet been identified. (8)

The neuropathology of PD is characterized by a selective loss of dopaminergic neurons that implies a degeneration of the *compact pars* of the substantia nigra (SN) and the consequent dopaminergic deficit in the striatum and other structures of the central nervous system (CNS) and brain tissue. (1,7)

Pathogenic mechanisms associated with genomic, epigenetic and environmental factors lead to conformational change. Consequently, due to the abnormalities in the ubiquitin-proteasome system and the deregulation of mitochondrial function and oxidate stress, a key protein, α-synuclein, is deposided. At the molecular level it stands

out for the phosphorylation of α-synuclein. These oligomers are neurotoxic, abnormal α-synuclein folds and forms insoluble fibrilles that are deposited in the cytoplasm of neurons (Lewy bodies) (LB) and neurites (Lewy neurites) (LN). (1,7)

The intracerebral formation of LB and LN begins at defined sites of induction and progresses in a topographically predictable sequence that is related to some extent to the stages of the disease. As the disease progresses, the autonomic, limbic, and somatomotor components are especially damaged. (9) It has been postulated that idiopathic PD is caused by an unknown pathogen (virus or bacteria) that enters the body through the nasal cavity that subsequently and once ingested, it reaches the intestine initiating Lewy pathology (LP) in the nose and intestine. For this reason, a proportion of patients may present prodromal hyposmia and constipation. This is part of Braak's hypothesis in which it also postulated a staging system that described the spread of LP (α-synuclein) from the peripheral nervous system to the central nervous system. (10)

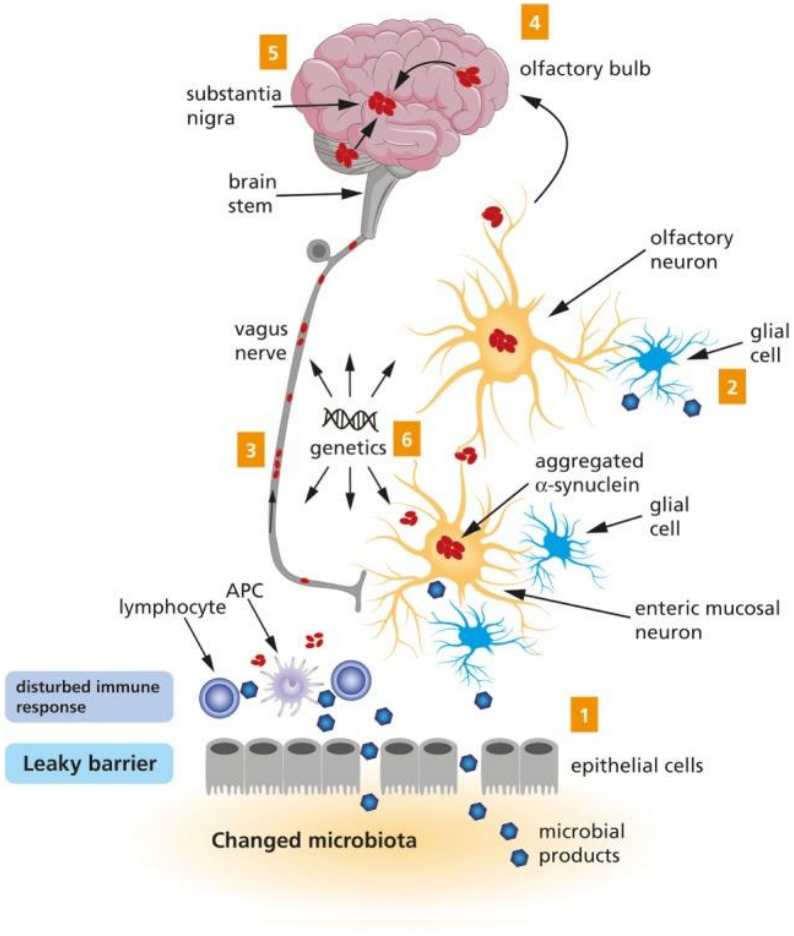


Illustration 1- Schematic representation of Braak's hypothesis of PD (10)

3.1.3. Clinical and prognosis

PD can be clinically defined by premotor (or presymptomatic) stages and motor or diffuse (or symptomatic) stages.

Classically it has been defined as a motor system disorder defined by the slow and asymmetrically onset of four cardinal symptoms (bradykinesia, rest tremor, stiffness and ultimately alteration of postural reflexes), although not all patients present these four symptoms simultaneously. But the reality is that PD is a complex systemic neurological disorder. In addition to the motor manifestations there can appear aswell cognitive, autonomic and sensory symptoms as we can observe in the *Illustration 2*. These non-motor manifestations are present in 97% of patients and can lead to a decrease in the quality of life of these patients.(3,11)

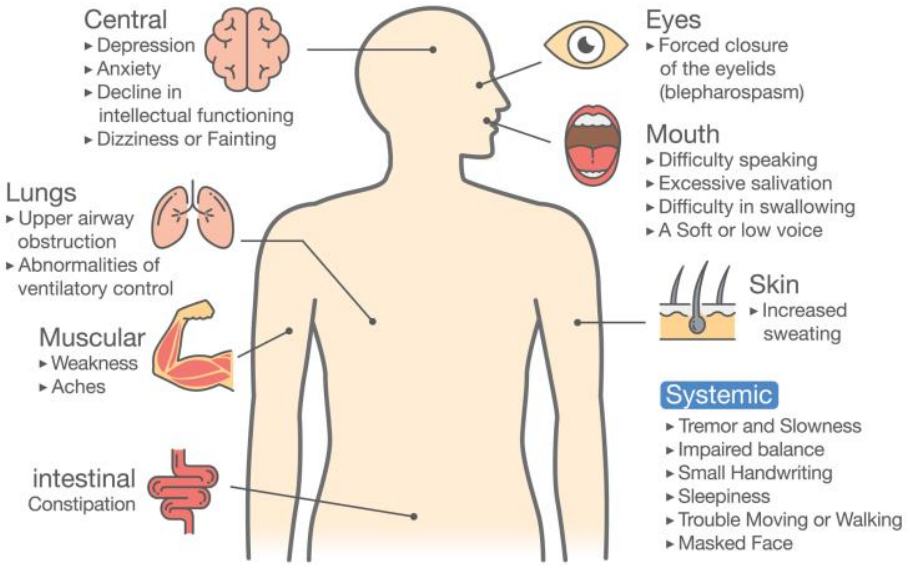


Illustration 2 - Symptoms of Parkinson's Disease

Knowing that we are dealing with a neurodegenerative disease without medical treatments that can slow the progression of the illness, we see that the clinical course of patients with PD shows a progressive deterioration, even though it presents with a heterogeneous evolution. This progression was seen in the initial studies of Hoehn and Yahr in which 25% of PD patients became severely disabled within 5 years, 67% within 10 years, and 80% within 15 years. The Hoehn and Yar stage classification it is used to study the motor evolutionary situation of PD. It should be remembered that PD is associated with an increase of mortality two to five times more than the general population by age with an average survival of 6 to 22 years. (3,12).

Table 3 - Hoehn and Yahr Stages (13)

Hoehn and Yahr Stages	
Stage 0	No signs of disease
Stage 1	Unilateral disease
Stage 1,5	Unilateral disease, with axial involvement
Stage 2	Bilateral disease, without affecting postural stability
Stage 2,5	Bilateral disease, with mild impairment of postural stability
Stage 3	Mild to moderate bilateral disease, with postural instability
Stage 4	Serious alteration; still able to walk without help
Stage 5	In a wheelchair or in bed

The following diagram correlates the clinic, the pathophysiology and the stages previously discussed.

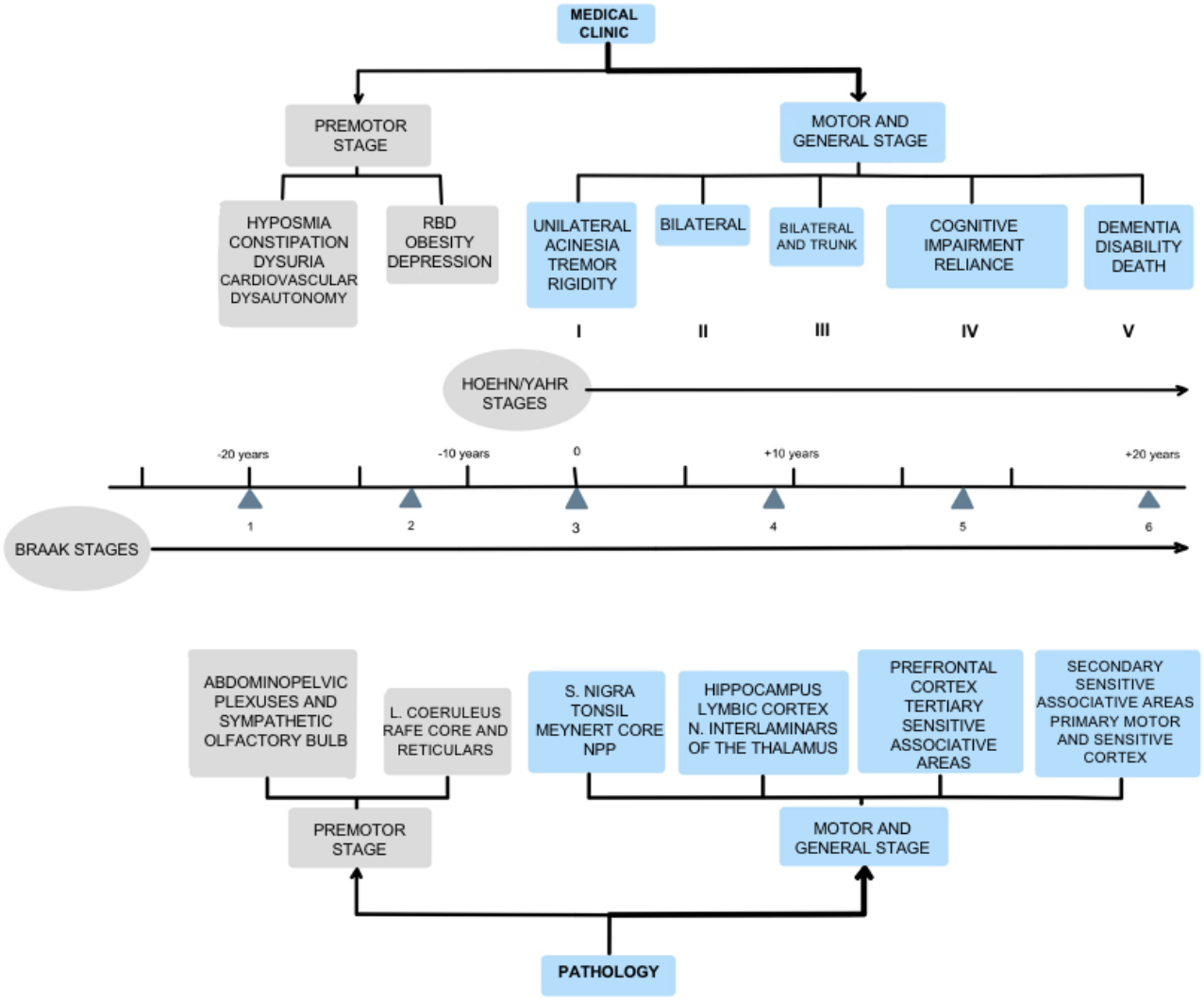


Illustration 3 – Scheme of possible clinical and pathological natural history of idiopathic PD (inspired by Braak's hypothesis). (1)

3.1.4. Diagnosis

The diagnosis of PD is basically clinical (and therefore we will diagnose patients when symptoms occur), its confirmation is by histology (and therefore a definitive post-morten diagnosis). We do not find physiological or blood tests that allow the diagnosis of the disease, and although we do find imaging tests, such as brain resonance, dat-scan and myocardial scintigraphy, used in usual clinical practice which allows us to observe very specific abnormalities of the disease. The possible study of CSF in these patients has also been considered as a possible diagnostic method in prodromal stages. Therefore, nowadays the conventional criteria defined by the United *Kingdom Parkinson's Disease Society Brain Bank,* which are very specific. These criteria despite having been created more than 25 years ago continue to be the most used today. The *Movement Disorders Society (MDS-PD)* has proposed new criteria aimed primarily for clinical research and are beginning to be used in daily clinical practice; these new criteria are adapted to both the prodromal and motor phase of PD. (3,14–16)

To diagnose a parkinsonism, the criteria require the presence of bradykinesia and at least one other classic motor symptom of PD (muscle stiffness, tremor at rest from 4 to 6 Hz or postural instability not caused by another primary dysfunction). Once parkinsonism is confirmed the diagnosis can be adjusted to determine if the patient has idiopathic PD or another parkinsonian form. To do so, we will rely on the support criteria or the exclusion criteria. (17)

The differential diagnosis of PD should include an anamnesis, reviewing of the patient's medical history, assesing symptoms through a complete physical examination and finally ruling out alternative diagnoses.(18)

Table 4 – United Kingdom Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria (1992) (17)

UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria (1992)
Diagnosis of Parkinsonian syndrome
· Bradykinesia (slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive actions) · And at least one of the following: - Muscular rigidity - 4-6 Hz rest tremor - Postural instability not caused by visual, vestibular, cerebellar, or proprioceptive dysfunction
Exclusion criteria for Parkinson disease
· History of repeated strokes with stepwise progression of parkinsonian features · History of repeated head injury · History of definite encephalitis · Oculogyric crises · Neuroleptic treatment at onset of symptoms · More than one affected relative · Sustained remission · Strictly unilateral features after 3 years · Supranuclear gaze palsy · Cerebellar signs · Early severe autonomic involvement · Early severe dementia with disturbances of memory, language, and praxis · Babinski sign · Presence of cerebral tumor or communicating hydrocephalus on CT scan · Negative response to large doses of levodopa (if malabsorption excluded) · MPTP exposure
Supportive prospective positive criteria for Parkinson disease **(Three or more required for diagnosis of definite Parkinson's disease)**
· Unilateral onset · Rest tremor present · Progressive disorder · Persistent asymmetry affecting side of onset most · Excellent response (70-100%) to levodopa · Severe levodopa-induced chorea · Levodopa response for 5 years or more · Clinical course of 10 years or more

3.1.5. Treatment

There are no drugs that modify the evolution of Parkinson's disease. The current treatment of PD is exclusively symptomatic. Advances in the future will focus on reducing or stopping neurodegeneration, symptomatic treatments for those symptoms that do not respond to L-DOPA, and finally treatments for those symptoms that greatly influence the patient's quality of life such as dysautonomic disorders or pain for example. (1)

Several different strategies are effective for the medical treatment of motor symptoms in PD but we do not find a clear consensus in favor of a single treatment strategy. Different algorithms are proposed depending on the stage of the disease and although it ends up being an individualized treatment, these algorithms can help in decision making. Dopaminergic treatment can relieve motor symptoms for a while but motor fluctuations may eventually appear, which is why we need to try to eradicate these fluctuations for as long as possible. (19) Regarding the treatment of non-motor symptoms of PD and despite knowing that they have a very important influence on the quality of life of patients, we find few evidence-based recommendations. (20)

There are several pharmacological groups that can be used for the symptomatic treatment of PD (in the *table 5* we can observe them) but we do not find a recommended drug to start treatment, taking into account factors such as the severity of symptoms, the ability to perform activities, the cost and preferences of the patient (we may even decide not to start treatment if the symptoms are very mild). So far the initial recommendation is to delay pharmacological treatment until the symptoms of PD significantly limit the patient's motor functions in order to avoid unnecessary side effects to the drugs of choice, but there is still debate about when to iniciate it.(21) L-DOPA achieves slightly better control than dopamine agonists and IMAO-Bs, but we need to keep in mind the dyskinesias and motor fluctuations associated with the drug that occur in 40-50% within the first five years of chronic treatment and 70-80% after 10 years of treatment. It is unlikely that patients receiving less than 400-500mg of L-DOPA will present these fluctuations. On the other hand, since dopaminergic agonists are less likely to develop these abnormal movements (dyskinesias) they have also been proposed as an initial treatment, although this advantage decreases over time.(22)

Currently, the treatment of motor fluctuations presented by patients with advanced PD has as purpose continuous dopaminergic stimulation. We also have surgical options, such as deep brain stimulation or intestinal gels of L-DOPA. (22,23)

Table 5 - Drugs used to treat Parkinson's disease (24)

Drugs that increase dopaminergic activity	**Dopamine precursors**	**Levodopa** (alone or in combination with dopa decarboxylase inhibitors: benserazide or carbidopa)
	Dopamine agonist	**Ergoline derivatives**: bromocriptine, lisuride, pergolide i cabergoline
		Non-ergoline derivatives: ropinirole, pramipexole i apomorfina
	Inhibitors of dopamine metabolism	**MAO B inhibitors (IMAO-B):** selegiline i rasagiline
		COMT inhibitors: entacapone
Drugs that decrease dopaminergic activity	**Central action anticholinergics**	Trihexyphenidyl, biperiden i procyclidine
Amantadine		

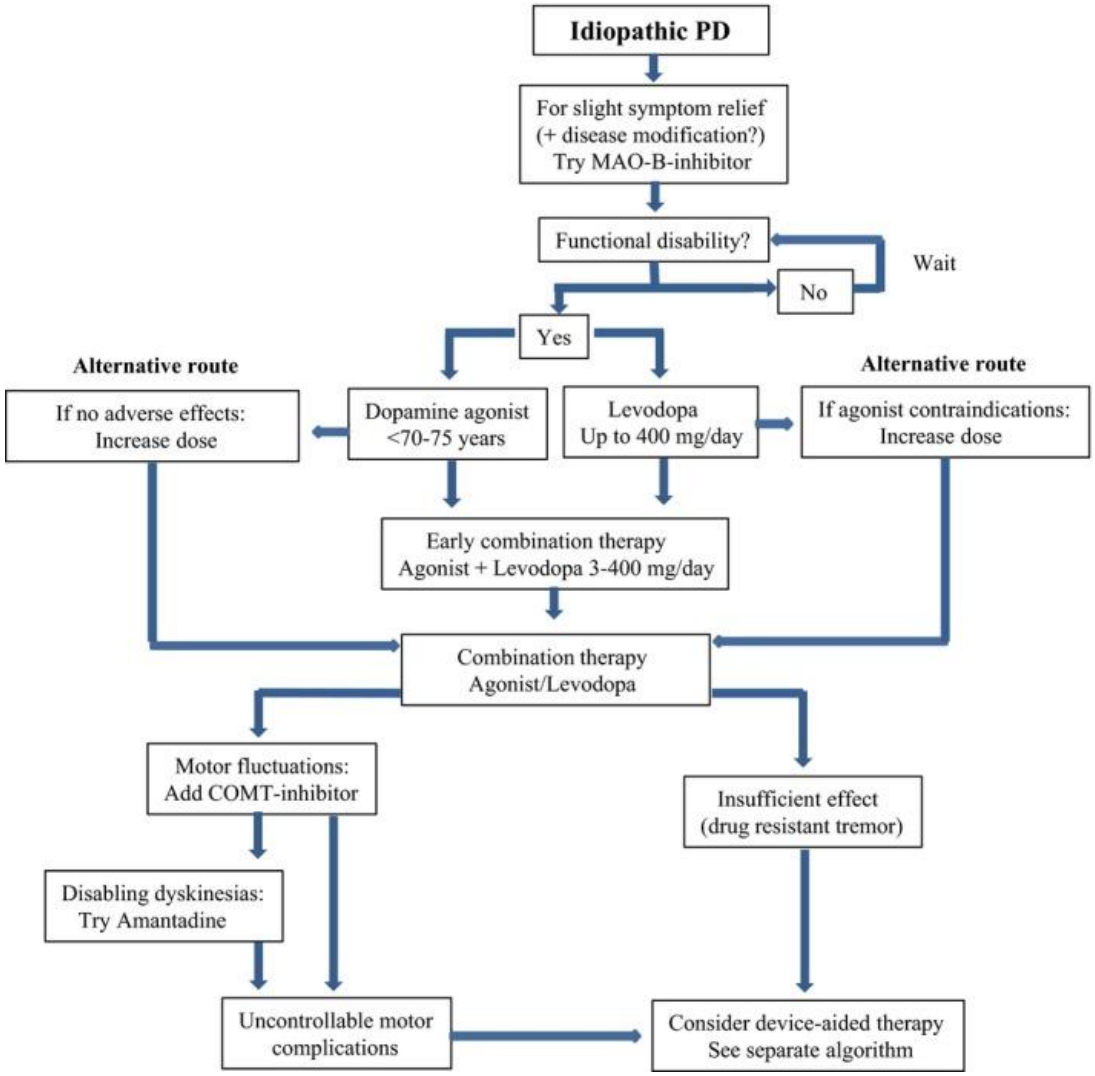
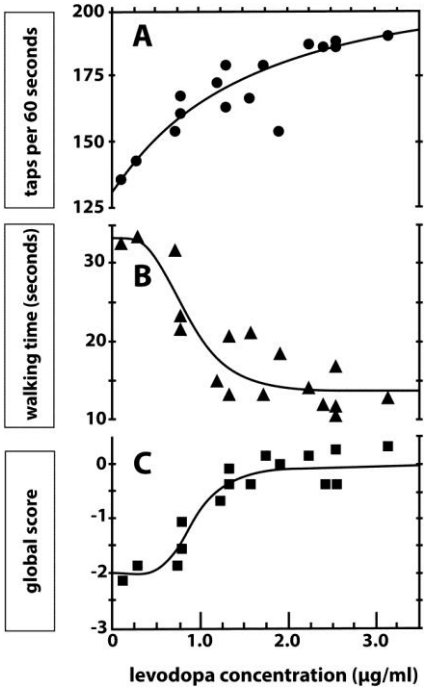


Illustration 4 - Algorithm for medical treatment of PD (19)

3.2. Levodopa

3.2.1. Pharmacodynamics and metabolic pathway



Levodopa (L-3,4-Dioxyphenylalanine) is the most effective metabolic precursor of dopamine and the most effective symptomatic drug for patients with PD, being its Gold Standard treatment, but it does not stop the evolution of the disease. It belongs to the pharmacotherapeutic group of dopaminergic agents dopa and dopa derivatives (with ATC code N04BA02 – Sinemet®- and ATC code N04BA – Madopar®-). This orally administered drug remains after almost 60 years of its introduction the main treatment option for Parkinson's disease. (3,25,26)

Illustration 5- Pharmacodynamic modeling of actual and modeled mean effect measurements versus mean plasma l-dopa concentration for eight PD subjects receiving two doses (at 4-hour intervals) of controlled-release CD-l-dopa 50-200 mg (Sinemet CR 50-200)

Dopamine has control over the response messages from certain regions of the brain that control muscle movement; when the amount of dopamine is scarce there is a difficulty in movement. Levodopa becomes dopamine by the enzyme dopa decarboxylase in the brain to reduce symptoms of PD. This drug is usefull for all commercially available L-DOPA formulations containing an AADC inhibitor (carbidopa -Sinemet®- o benzerazida -Madopar®-).

Carbidopa or benzerazide, inhibitors of the enzyme dopa decarboxylase, prevent the metabolism of levodopa to dopamine in extracerebral tissues, ensuring that a higher proportion of levodopa arrives to the brain. It also manages to reduce the required doses of L-DOPA, thus reducing the extracerebral side effects associated with the drug. The ratio of carbidopa to levodopa is currently 1:4 due to its pharmacokinetic advantages and the decrease in adverse effects, although dosages are also presented in proportions of 1:10. (25,27,28).

In some patients with PD (especially in the early years after the initiation of L-DOPA treatment), relief of parkinsonian signs and symptoms persists without fluctuations regardless the increase and decrease in circulating drug concentration. Symptomatic control in these circumstances is called 'honeymoon', making therapeutic optimization easy to achieve. Despite this, two or more years after the introduction of levodopa, this response pattern is usually replaced by motor fluctuations that occur regularly during a cycle corresponding to the half-life of plasma clearance of the drug (about 3 hours). It should be remembered that L-DOPA does not improve some of the Parkinson's symptoms that appear and worsen during the course of the disease such as gait blockage, postural instability, autonomic dysfunction and dementia. (3,29)

Based on observations of levodopa's treatment results, two pharmacodynamic patterns have been discerned after drug administration: short-term response (STR) and long-term response (LTR). Both patterns can manifest themselves in the same patient. STR could be defined as the direct translation into the clinical effect of levodopa administered to the striatum, while LTR does not appear to follow the plasma concentration of the drug and remains for hours or days after discontinuing the drug. (29–31) Motor and non-motor fluctuations develop in up to 75% of patients after 4-6 years of treatment with L-DOPA, having a negative impact on patients' quality of life. (32)

3.2.2. Pharmacokinetics

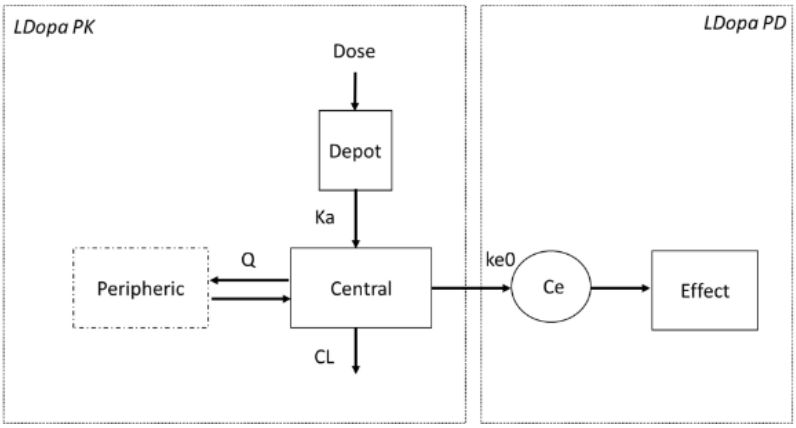


Illustration 6 - Schematic PK/PD model for levodopa. Ka: absorption rate constant; CL: clearance; Q: intercompartmental clearance; ke0: effect rate constant; Ce: effect compartment concentration (33)

- **Absorption**

Absorption of L-DOPA occurs in the proximal third of the small intestine (duodenum/ jejunum) and not in the stomach. (34) This absorption depends on the motility of the stomach (varies with diet and fasting) and gastric emptying which is erratic and may be delayed in PD, leading to an unpredictability of the levodopa's time concentration curve. The effects of L-DOPA on motor function depend on gastric emptying in patients who are in advanced stages of the disease. (35,36)

In oral administration of L-DOPA, intestinal absorption is rapid and complete but the bioavailability is <1% in the absence of any AADC inhibitors (benserazide or carbidopa), which is why efforts have been made to improve the bioavailability of the drug through new and more effective oral formulations or through innovative pathways of administration.(26,35)

Table 6 - Levodopa formulations and routes of administration alternative to standard levodopa tablets (33)

Route of administration and formulation	Main differences in pharmacokinetics compared with standard levodopa
Enteral (duodenal/jejunal) infusion	↓CV
Intravenous infusion	↓CV
Nasal	NA
Oral dual release	↓ tmax, ↑Cmax, ↑ AUC compared with sustained-release formulation
Oral liquid	↓tmax
Oral sustained realease	↑tmax, ↓Cmax, ↓CV
Pulmonary inhalation	NA

AUC = area under the plasma concentration-time curve; **Cmax** = peak plasma concentration; **CV =** coefficient of variation of plasma concentration (standard derivation divided by the mean plasma concentration); **NA** = not available;; **tmax**= time to reach Cmax; ↓ indicates decreased; ↑ indicates increased;

Therefore, the time it takes to reach the Cmax (t max) may vary between patients and within the patient, but it is generally reported to be approximately between 30 minutes to 2 hours. The Cmax and AUC of the levodopa increase proportionally with the dose.(27,37)

Differences have been reported regarding bioavailability depending on the sex of the patient, showing greater bioavailability in women compared to men of the same age. Differences in a reduction in oral clearance of the drug have also been described (these differences are suggested depending on the activity of the enzyme COMT). (38)

- **Distribution**

Levodopa has a saturable facilitated transport of plasma to the brain through the blood-brain barrier by the same LNNA system (neutral amino acid transport system) that operates in the intestinal mucosa. (38). Levodopa does not bind to plasma proteins and its distribution volume is 57 liters. The AUC of the levodopa in cerebrospinal fluid is 12% of the AUC in plasma. (37)

It has been proven that amino acids such as tirosine, phenylalanine, tryptophan, leucine, and valine competitively inhibit the transport of L-DOPA membrane, demonstrating a competition between the L-DOPA and the amino acids for uptake through the BBB.(39)

- **Metabolism**

Levodopa is metabolized by two main pathways (decarboxylation and o-methylation) and two secondary forms (transamination and oxidation).

The decarboxylation of levodopa to dopamine begins rapidly in the gastrointestinal tract by the L-amino decarboxylase acid enzyme (AADC) resulting as degradation products the homovanillic acid and dihydroxyphenylacetic acid. Only 30% of the dose reaches systemic circulation. Even high doses of an AADC inhibitor do not completely inhibit the extracerebral AADC, they allow to triple the amount of oral levodopa that

reaches circulation, preventing the peripheral metabolism of levodopa, thus improving bioavailability for the brain and reducing peripheral dopaminergic side effects such as nausea or vomiting. (26,37,38,40)

We find a rapid metabolic elimination in the presence of AADCI, the metabolism of levodopa moves towards the formation of 3-O-methyldopa by the enzyme COMT (catecol- O- methyltransferase) which has more activity in the liver and kidney. This metabolite has a 15-hour elimination life. (38) .

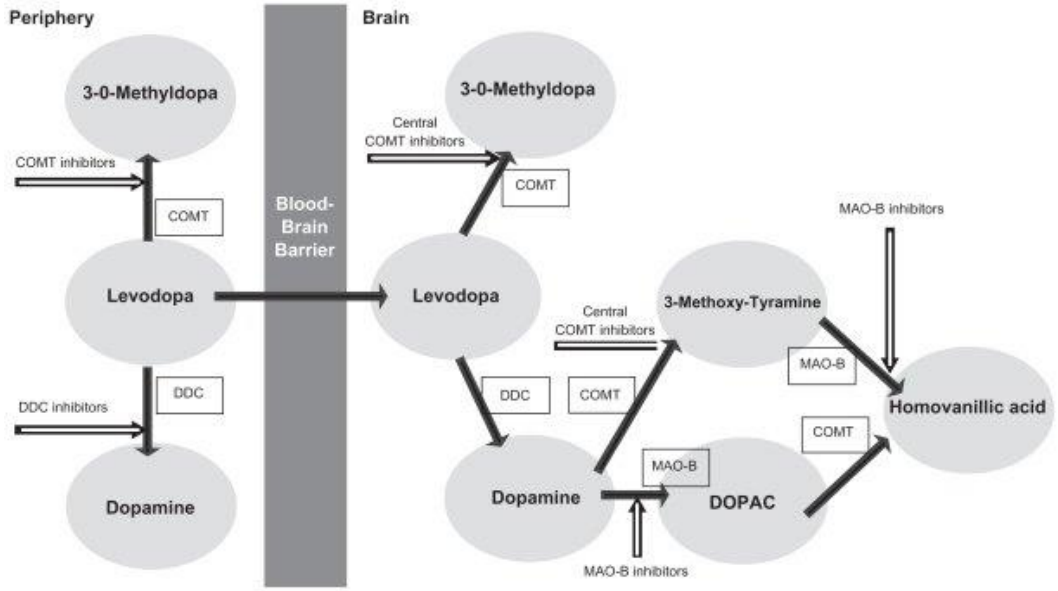


Illustration 7 - Metabolic pathways of levodopa**. Abbreviations:** COMT, catechol-O-methyltransferase; DDC, dopa-decarboxylase; DOPAC, 3,4-dihydroxyphenylacetic acid; MAO-B, monoamine oxidase type B. (41)

- **Excretion**

The elimination half-life (t (1/2)) of plasma levodopa is approximately 1.5 hours in the presence of an AADC inhibitor, and one hour in its absence. (28) A COMT inhibitor can also be added to further increase the t(1/2) of the L-DOPA. Plasma clearance is approximately 430 ml/min. Less than 10% of levodopa is excreted unchanged by the kidneys.(37)

It has been shown that the increase of the age magnifies statistically significantly the t(1/2) of levodopa and decreases the apparent oral clearence, reaching up 25% more availability of systemic levodopa in the elderly people, although it presents little clinical relevance. (38)

Intravenous or enteral infusions (duodenal/jejunal) produce stable concentrations of levodopa and motor performance. The enteral administration pathway is feasible in the long term in patients with severe fluctuations. (35)

Table 7 - Factors that influence the pharmacokinetics of orally administered levodopa in combination with an amino acid decarboxylase inhibidor (35)

Factor	Cmax	tmax	AUC	t1/2
Slowed gastryc emptying	↔, ↓	↑	↓	NA
Food (fat and LNAAs)	↓	↑	↓	↔
Advanced age	↑	↔	↑	↑
Low bodyweight	↑	NA	↑	NA
Sex (women vs men)	↔	↔	↑	↔
COMT-inhibitors (entacapone and tolcapone)	↓, ↔, ↑	↔	↑	↑

AUC = area under the plasma concentration-time curve; **Cmax** = peak plasma concentration; **COMT** = catechol-*O*-methyltransferase; **LNAAs** = large neutral amino acids; **NA** = not available; **t1/2** = elmination half-life; **tmax**= time to reach Cmax; ↓ indicates decreased; ↑ indicates increased; ↔ inidicates unchanged

3.2.3. Initial dosage

This study protocol selects those patients who were not previously being treated with levodopa as can be seen in section 7.3.

The AEMPS establishes two ways to define the initial doses these patients will receive. (25)

- For patients who begin treatment with Sinemet® 25mg/250mg tablet, the initial dose is half a tablet one to three times a day. It may occur that the optimum dose of carbidopa is not provided. If necessary, half a tablet can be increased per day or on alternate days until an optimal response is obtained.
- For patients who begin treatment with Sinemet® 25mg/100mg tablets, the initial dose is one tablet three times a day. The dose can be increased to one tablet daily or on alternate days as needed until an optimal response is obtained, up to an equivalent dose of eight tablets a day.

Dose response may be observed in one day, and sometimes in just one dose. Nevertheless, fully effective doses are usually achieved in seven days in contrast to weeks or months using levodopa alone.

In patients with Madopar® it is recommended to start treatment with a quarter of a tablet, three to four times a day. Once the tolerability of the initial dosage is achieved, the daily dosage can be increased slowly according to the patient's response (every 3-7 days). (37)

3.2.4. Adverse effects

Adverse reactions that occur frequently in patients with levodopa treatment are due to the central neuropharmacological activity of dopamine, and can usually be reduced by decreasing the dose of the drug. (25)

Although levodopa improves parkinsonian symptoms with a dose-related response, the drug was found to induce motor complications after a period of chronic use. Therefore, the most common adverse reactions are levodopa-induced dyskinesias (chorea, athetosis, ballism, dystonia, myoclonus, tics, sterotyped movement disorders or a combination of all of them) and motor fluctuations; these are known as late

complications to dopatherapy. The risk of developing motor complications to the drug is 40% to 4-6 years. (42,43)

Other side effects to the drug include digestive problems (especially nausea and vomiting), postural hypotension, sleep disturbances, involuntary movements and psychiatric alterations (dopamine dysregulation syndrome or impulse control disorder). The technical data sheets of the drug provided by the AEMPS accurately detail the possible adverse reactions that may appear. (25,37)

3.3. Vitamin B6 and its deficit

3.3.1. Definition

Vitamin B6 (pyridoxine) is one of the main molecules in the cells of living organisms. It is present in multiple foods dissolved in water such as meat, fish, nuts, cereals, fruits and vegetables. This vitamin is also present in commercialized multivitamin complexes and also in energy bars and powders. (44)

Vitamin B6 participates as a cofactor in more than 100 enzyme reactions such as reactions of carbohydrate metabolism, amino acids (especially homocysteine), glycogenesis, glycogenolysis, and lipid metabolism. Vitamin B6 also plays an initial role in the synthesis of porphyrins and participates in the essential functioning of cells. Speaking of cognitive development it plays an important role in the synthesis of neurotransmitters (enzymatic decarboxylation from DOPA to dopamine, the conversion of tryptophan to nitric acid and serotonin and the conversion of glutamic acid into GABA), immunological function and production of interleukin-2 and the formation of hemoglobin. (44)

3.3.2. Metabolism

Vitamin B6 has three natural forms: pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM) all of which are transformed into their active forms in the body, which is 5-phosphate pyridoxal coenzyme (PLP or P5P). (45,46)

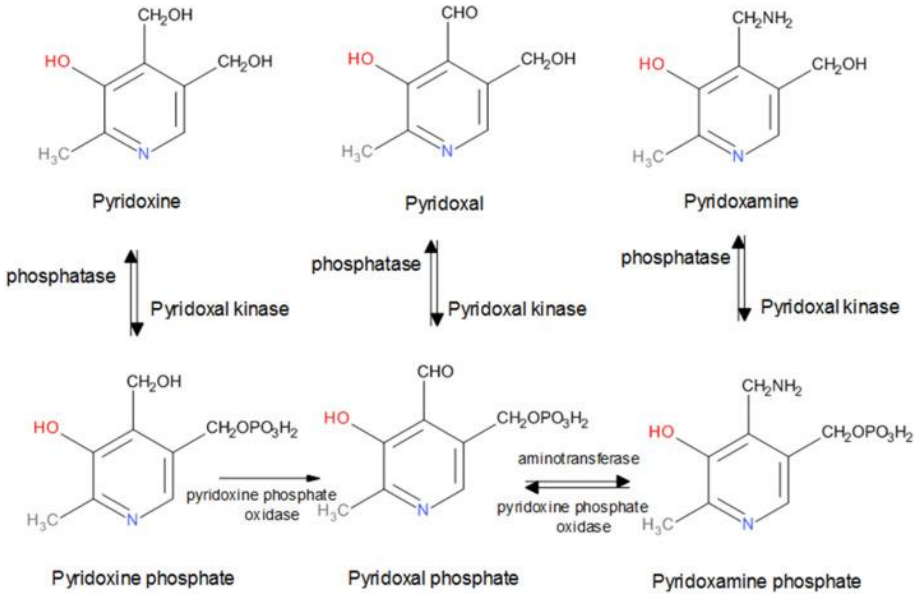


Illustration 8 - Vitamin B6 metabolism. (46)

Pyridoxine is predominantly absorbed in the jejunum by passive diffusion and metabolized at a cellular level by the liver to its active form PLP. After the PLP hydrolysis the pyridoxal phosphate is available for all cells in our body. This vitamin is transported by albumin.

Most pyridoxal that exceeds tissue requirements is oxidized by the liver to 4-pyridoxic acid (4-PA) which is the main degradation product of vitamin B6 in urine. Its excretion occurs in the kidney, the semi-life elimination is between 15 to 20 days. (44,46)

3.3.3. Epidemiology

The human body cannot store this vitamin; this is why it requires a daily intake. Risk factors related to presenting altered pyridoxine values may include excessive or inadequate intake.

Vitamin B6 recommendations are made according to the age and stage of life of the patient. In adults it is recommended an intake between 1-1,7 mg per day. During fetal brain development and childhood the body requires an adequate amount of vitamin B6. (44)

3.3.4. Etiology and pathophysiology

Isolated B6 deficiency is rare and usually appears in combination with other B-Complex deficiency. Pyridoxine deficiency can be related to a low intake of the vitamin (although this is rare because, as we have mentioned, it is found in almost all foods), due to insufficient gastrointestinal absorption (such as inflammatory bowel disease, coeliac disease or small bowel surgery), liver dysfunction, protein-energetic malnutrition and pharmacological interaction or antagonism of PLP metabolism. (46,47)

Table 8- Risk factors for piridoxine deficiency (47)

Conditions that increase risk for PN deficiency	
Advanced age	
Medical conditions	Severe malnutrition Hospitalization Coeliac disease Hepatitis and extrahepatitic biliary obstruction Hepatocellular carcinoma Chronic renal failure Kidney transplant Hyperoxaluria types I and II High serum alkaline phosphatase level such as in cirrhosis and tissue injury Catabolic state
Medical procedures	Hemodialysis Peritoneal dialysis Phototherapy for yperbilirubinaemia
Social-behavioral conditions	Excessive alcohol ingestion (except for pyridoxine-supplemented beer) Tobacco smoling Severe malnutrition
Other risk factors	Poisoning, such as Gyromitra mushroom poisoning Perinatal factors, such as pyridoxine-deficient mother Inherited conditions, such as pyridoxine dependent neonatal seizures
Other patient	Sideroblastic anemia Pregnancy Physical exercise

Drug- interaction	Mechanism of action
Isoniazid (hydrazines)	Reacts with PL and PLP
Cycloserine	Reacts with PLP, forms oxime
L-3,4-Dihydroxyphenlalanine derivative	Reacts with PLP, forms tetrahydroquinoline derivative
Penicillamine	Reacts with PLP, forms thiazolidine
Ethinylestradiol, mestranol	Increased enzyme levels and retention of PLP in tissue
Ethanol	Increased catabolism of PLP
Theophylline, caffeine	Inhibition of pyridoxal kinase

3.3.5. Clinical

This deficiency has been linked to an increased risk of cardiovascular diseases and polyneuropathy (weakness, numbness and pain). On the other hand, vitamin B6 supplementation in relatively high doses has also been associated with polyneuropathy, which is why the *European Food Safety Authority (EFSA)* has reduced the upper limit of this vitamin to 25 mg/day to ensure its safety. (46,47)

Vitamin B6 deficiency may occur in the form of resistent seizures to antiepileptic drugs in young patients. In adults, severe deficiency can occur in the form of skin rashes and changes in mental status. However, this vitamin in excessive doses (more than 100mg/dL) and administered chronically (months to years) may present toxicity in the form of sensory neuropathy (decreased sensation of touch, temperature and vibration) and movement disorders, photosensitivity, gastrointestinal symptoms. (44)

3.3.6. Relationship with the PD. Role in the metabolic pathway of L-DOPA

B-complex vitamins are consumed in the metabolic process of levodopa, in which homocysteine is formed. This homocysteine suffers a process of re-methylation and forms methionine. This process requires cobalamin (vitamin B12) and B9 (folate). The homocysteine can also be metabolized into cysteine, requiring pyridoxine (vitamin B6) as a cofactor. Therefore B12, B9 and B6 act as methyl group donors and when these substrates run out, homocysteine accumulates. (48)

As a result, when the dose of levodopa is increased, the levels of B12, B9 and B6 decrease and the levels of homocysteine increase. In addition, carbidopa binds irreversibly and permanently deactivates free pyridoxine and enzymes dependent on it. (49)

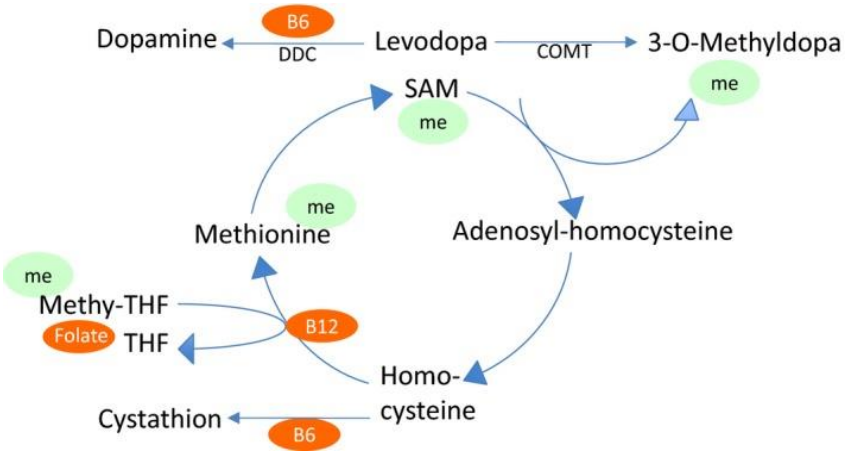
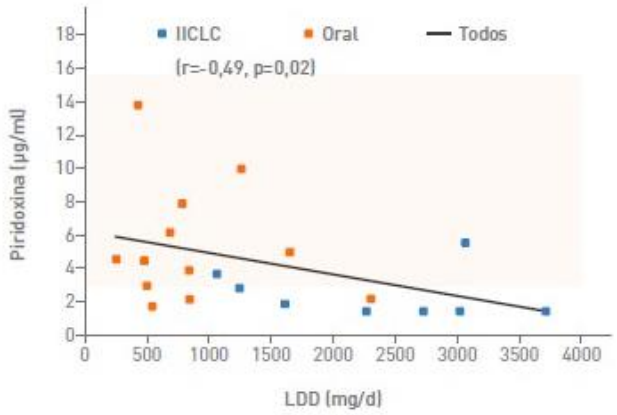


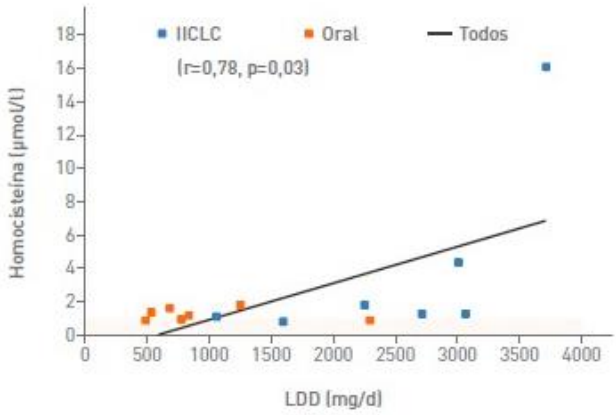
Illustration 9 - Levodopa metabolism within the plasma.(B6 =pyridoxine, B12 = cobalamin, COMT = catecholamine-O-methyl-transferase,DDC = dopamine decarboxylase, me = methyl group transfer, Methyl-THF = methyltetrahydrofolate, SAM = S-adenosyl methionine). (49)

It is suspected that vitamin B12 and folate deficiency could be responsible for the development of neuropathy in patients with PD, and pyridoxine deficiency may also be related. (49)

The daily dose of levodopa correlates significantly with the reduction of pyridoxine levels (p=0.02) in all patients regardless of the drug administration route, as well as increased levels of homocysteine but only in those patients receiving the drug through intestinal infusion. No statistically significant results were found for cobalamin and folate. (49)

Table 9 - Correlations between plasma levels of pyridoxine (A) and homocysteine (B) with the daily dose of levodopa (LDD)**.** (48)





4. JUSTIFICATION

Parkinson's disease currently remains a progressive and incurable disease. The doctors have spent decades using the same drugs to treat these patients. New ways of administration have been formulated, new combinations searching better results, among others; but the basis of the treatment remains the same, favoring the dopaminergic system in order to improve the patient's clinic.(1,3,26,49)

Symptomatic treatment is the solution given to these patients. Different medical options can be offered to patients with Parkinson's disease. Some of these options include introducing IMAO-B and wait to introduce other drugs in patients with little functional impairment, as well as introducing drugs that increase dopaminergic activity, such as dopaminergic agonists or levodopa, according to the age of the patient and his clinic. Despite this algorithm, the reality is that levodopa remains the most effective treatment and drug of choice.(1,3,19,26)

We know that several vitamins from complex B such as cobalamin (B12), pyridoxine (B6) and folate (B9), as well as other biomolecules such as homocysteine, are involved in the metabolic pathway of L-DOPA. Hypotheses have been formulated about the relationship of the daily dose of L-DOPA and the drop in pyridoxine levels. (48,49) In contrast, we find very little information that studies the clinical impact of the altered levels of these vitamins in these patients.

The neurology team specialized in movement disorders at *Hospital Universitari Doctor Josep Trueta* and *Hospital de Santa Caterina* has observed a number of clinical cases in which a significant percentage of patients who had gastrointestinal intolerance to recently indicated levodopa showed low pyridoxine levels. These series of observed clinical cases has led them to start a pilot trial to measure the impact of the administration of pyridoxine in reducing these adverse symptoms. Currently, however, we do not have studies that firstly demonstrate the relationship between the levels of these biomolecules as possible risk factors in the appearance of gastrointestinal adverse effects in the levodopa.

The little current evidence that we find in relation to the subject makes it necessary for us to conduct a study on the relationship that exists between the levels of pyridoxine as a possible risk factor in the appearance of gastrointestinal adverse effects to L-DOPA. In spite of everything, the mechanisms by which low levels of pyridoxine can lead to gastrointestinal intolerance to the drug remain still unknown. New hypotheses will be necessary to solve this question.

To obtain the first scientific evidence that shows this possible relationship, we will need to carry out observational analytical studies that allow us to answer whether or not the association seen by the neurologists on the daily clinical practice, and explained before, exists and if it is statistically significant.

5. HYPOTHESIS

5.1. Main hypothesis

Low levels of vitamin B6 are associated with an increased risk in the onset of gastrointestinal intolerance to oral L-DOPA in patients with Parkinson's disease.

5.2. Secondary hypothesis

Levels of vitamin B12, folic acid and/or homocysteine are associated with an increased risk in the appearance of gastrointestinal intolerance to oral L-DOPA in patients with Parkinson's disease.

6. OBJECTIVES

6.1. Main objective

To assess the risk associated between vitamin B6 levels with the appearance of gastrointestinal intolerance to oral L-DOPA in patients with Parkinson's disease in Catalonia.

6.2. Secondary objectives

The secondary objectives of this study are:

- To study whether there is an association between levels of vitamin B12, folic acid and/or homocysteine with the risk of gastrointestinal intolerance to oral L-DOPA in patients with Parkinson's disease in Catalonia.
- To assess the percentages and profile of adverse reactions to oral L-DOPA.

7. METHODOLOGY

7.1. Study design

This study will be developed as a 3-year prospective observational cohort study (2020-2022) with the objective to study the risk factors associated with the appearance of gastrointestinal intolerance in patients with Parkinson's Disease in Catalonia who are treated with oral L-DOPA.

7.2. Study setting

The study will be designed to be multicenter.

Patients will be offered by their reference neurologists the possibility to be included in the study during the neurology consult at the time they are prescribed L-DOPA as a new treatment guideline.

The participants who will be part of the study will be obtained from the different neurology services of the reference hospitals in Catalonia diagnosed with Parkinson's disease, as long as they meet the inclusion and exclusion criteria.

The reference hospitals that will participate in this study are:

Table 10 - Study Reference Hospitals

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

The data collection will be recollected at the hospital on the follow-up visits that these patients have scheduled on a regular basis with their specialists of reference.

A researcher will be appointed as a representative of each different hospital, in order to achieve a more fluid communication.

7.3. Study population

The target population of our study (both the exposed and the non-exposed cohort) will include those patients diagnosed with Parkinson's disease and visited continuously in the specialized consultations of movement disorders from the neurology services at the different reference hospitals participating in the study mentioned above, who initiate treatment with L-DOPA for clinical reasons and who agree to be part of the study.

In the table below we can observe the criteria that the population requires to be part of the study:

Table 11 – Study selection criteria

Inclusion criteria	Exclusion criteria
- Patients diagnosed with Parkinson's disease. - In those who start treatment with L-DOPA orally for clinical reasons. - Do not show gastrointestinal intolerance before starting the study. - They can take other concomitant pharmacological groups for PD (IMAO-B as an example). - That they have signed the informed consent.	- Patients diagnosed with **other parkinsonian syndromes** (PSP, multiple system atrophy). - Patients who started the treatment **more than 3 months ago.** - Patients on concomitant treatment with **dopamine agonists** (DA). - Patients with **previous digestive pathology** who have a clinical similar to gastrointestinal intolerance. (H Pylori, GERD, malabsorption syndrome, ...) - Patients being treated with other **drugs** that may have **similar adverse effects** to those studied. - Patients with any **psychiatric disorder or mental retardation** that impede proper understanding of the study.
Withdrawal criteria	
- Treatment is not effective to control the symptoms and requires other treatments. - During the study period start taking medication that may have similar side effects. - During the study period is diagnosed with any digestive pathology that may cause gastrointestinal symptoms. - Do not participate in the follow-up of the study.	

The cohort will be defined by all the patients who meet the inclusion criteria and none of the exclusion criteria from the table above. Once we have our initial cohort defined based on the selection criteria, we will study its exposure to the risk factor that we are studing, pyridoxine, thus leading to a division of the cohort into two groups that will be:

- Group 1. **Cohort exposed to the RF**. Defined as those patients who meet the selection criteria and present **low pyridoxine levels**.
- Group 2. **Cohort NOT exposed to RF.** Defined as those patients who meet the selection criteria and present **normal or high levels of pyridoxine.**

7.4. Subject selection

The specific subjects of our study will include those patients who have met the selection criteria.

7.5. Sampling

We will use a consecutive non-probabilistic sampling method. L-DOPA will not be given randomly. It will be given based on clinical reasons when the doctors feel it is appropriate.

7.5.1. Sample selection

As new patients meet the requirements based on the selection criteria of the study, they will be proposed to be part of it. The time interval in which new patients can be added to the study will go from February 2021 to January 2022 (1 year), corresponding to phase 3 of the timeline, section 11 – patient inclusion and data collection –. Therefore, the last dates on which the necessary analyzes will be performed to study the independent variable and the covariates it will be no later than January 2022 as well. The dependent variable will be measured from May 2021, date corresponding to the first patients evaluated in the follow-up visit 3 months after their inclusion in the study, until the end of phase 3 corresponding to April 2022. .

7.5.2. Sample size

We do not find similar studies to perform the calculation of the sample size. For this reason, we have considered the study as a pilot study.

Accepting an alpha risk of 0.05 and a beta risk lower than 0.2 in a bilateral contrast, 70 subjects are needed in the exposed group and 210 in the non-exposed group to detect a minimum relative risk of 2 if the patient rate in the group of non-exposed is 0.2. A follow-up loss rate of 10% has been estimated. POISSON approximation has been used. All this data was calculated using the GRANMO sample size calculator.

7.6. Variables and measurement methods

Table 12 - Variables of the study

VARIABLE	TYPE	CATEGORY OF VALUES
Independent variable		
Pyridoxine levels (vitamin B6)	Continuous quantitative	Numerical
Dependent variable		
Gastrointestinal intolerance	Dichotomic nominal qualitative	Yes / No
Covariates		
Gender	Dichotomic qualitative	Men / Women
Age	Discrete quantitative	Numerical
Dose of levodopa	Continuous quantitative	Numerical
Cobalamin levels (vitamin B12)	Continuous quantitative	Numerical
Folic Acid levels (vitamin B9)	Continuous quantitative	Numerical
Homocysteine levels	Continuous quantitative	Numerical

***All data related to pyridoxine and covariates cobalamin, folic and homocysteine, involved in the metabolic pathway of levodopa have been obtained in collaboration with the clinical analysis services of the Hospital de Figueres and the Hospital de Santa Caterina.*

7.6.1. Dependent variable: Gastrointestinal Tolerance to L-DOPA.

- **Type of variable.**

  It is a **dichotomic qualitative variable** (gastrointestinal intolerance YES / gastrointestinal intolerance NO).

- **Definition.**

  The "*Agencia Española del Medicamento y Productos Sanitarios"* exhaustively details the types of adverse reactions that this drug can cause. (25,37) Gastrointestinal disorders are listed in the table below:

Table 13 - Gastrointestinal disorders to L-DOPA

Gastrointestinal disorders to L-DOPA	
Included in the gastrointestinal tolerance scale to L-DOPA	**Not included in the gastrointestinal tolerance scale to L-DOPA**
- Nausea - Vomiting - Dyspepsia - Abdominal pain and digestive discomfort	- Diarrhea - Dysphagia - Flatulence - Constipation - Dry mouth - Gastrointestinal bleeding - Appearance of duodenal ulcer - Dark saliva - Burning sensation of the tongue Sialorrhea

** Adverse effects not included in the gastrointestinal tolerance scale have been based on routine clinical practice (characteristic symptoms of PD, need to be assessed by a different specialist, or because they would not fit within the concept of gastrointestinal intolerance).*

- **Study method.**
  This variable will be collected using a scale-based data collection sheet (Annex 4). Gastrointestinal tolerance to L-DOPA will be evaluated directly by the patient during the period of the study based on a ***subjective evaluation.*** The patient will assess the presence of clinical symptoms included in the **gastrointestinal tolerance scale** based on a **dichotomic scale** (No=0 points -symptom not present- // Yes=1 point -symptom present-) (Annex 4). A score ≥1 will define the existence of gastrointestinal intolerance to this drug. The researcher will collect the final score of the scale and record it in the data base.

Next, we will define the gastrointestinal disorders that may appear while taking L-DOPA detailed by the AEMPS included in the scale of gastrointestinal tolerance to the drug to have a frame of reference. This previous step subsequently helps us to develop the dichotomic scale of subjective evaluation.

- **Nausea:** described as the strong and unpleasant desire to vomit, which the patient can define as "symptoms of dizziness" or feeling "sick to the stomach" and other possible accompanying symptoms. (50)
- **Vomiting:** described as the violent expulsion of stomach contents through the mouth. (50)
- **Dyspepsia:** colloquially known as gastritis or indigestion is defined as pain or discomfort in the upper abdomen. Its symptoms can be pain, distention, swelling, burning sensation or a feeling of being filled very quickly with little food. (51)
- **Abdominal pain and digestive discomfort:** defined as an unpleasant sensation, such as pain or discomfort, which the patient may feel related to food ingestion, their pass through the bowel or bowel movements. (52)

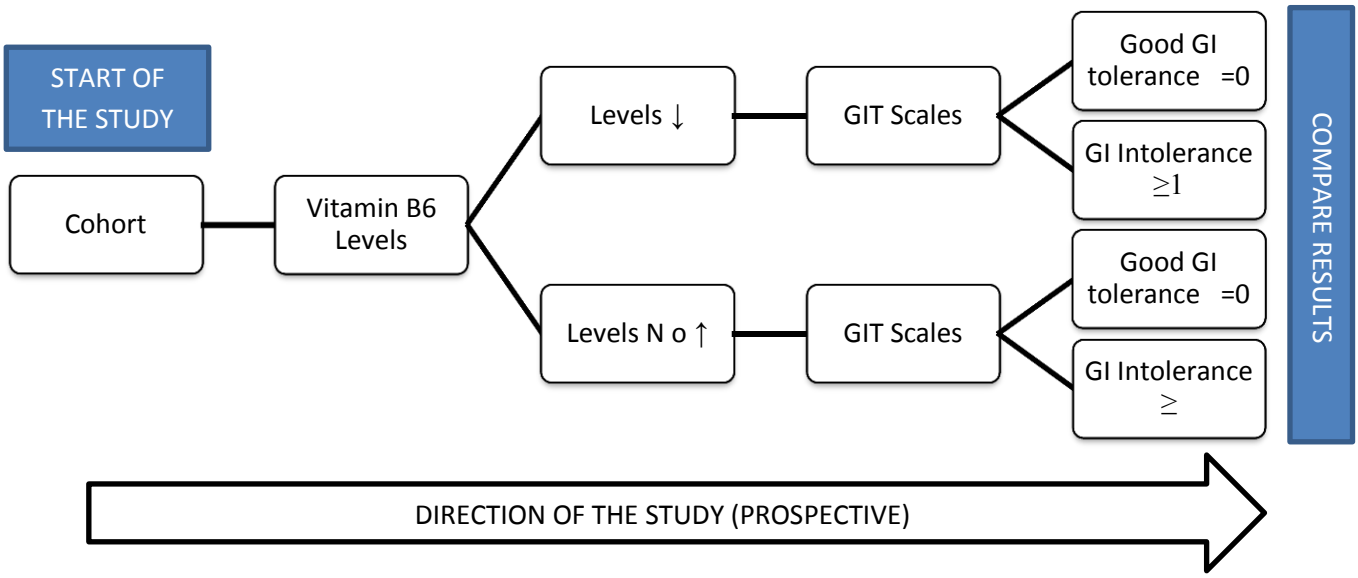
7.6.2. Independent variable: Vitamin B6 levels.

- **Type of variable**
  It is a continuous quantitative variable.
- **Definition and functions** (section 3.3)
- **Study method.**
  High Performance Liquid Chromatography - Fluorescence Detector.
- **Trademark.** Thermo Fisher Scientific®
- **Sample.** 1 mL of serum (2-8º - Refrigerate).
- **Transport.**
  Protect from light. The refrigerated sample is stable for 48 hours. Freeze the sample if it is received in the laboratory more than 48 hours after its collection.
- **Reference Values (RV).**
  RV: 20.00 – 121.00 nmol/L (* 0.247158 =ng/mL).
- **Coefficient of Variation (CV).**
  7.96%

Table 14 - Vitamin B6 reference values

Reference values vitamin B6		
Low	**Normal**	**High**
<4.94 ng/mL	4.94 ng/mL – 29.91 ng/mL	>29.91 ng/mL

** With regard to our study we will group normal values and high values of vitamin B6 into one group*



7.7. Covariates

In order to avoid as much as possible the confounding variables that may interfere with the results of the study, firstly the doses of the drug prescribed by its specialists will be noted and secondly, other biomolecules participating in the metabolic pathway of the LDOPA will be studied.

7.7.1. Gender and age

7.7.2. Dose of L-DOPA

- **Type of variable.**
  It is a continuous quantitative variable**.**
- **Reason of selection.**
  It is proposed as a possible confounding variable because part of the adverse drug reactions can be dose-dependent. At the beginning of the disease, a clear relationship between the dose of levodopa, the coincidental plasma profile and the antiparkinsonian effect cannot be seen. (38) The dose of levodopa has also been linked to a decrease in B-complex vitamin levels. (48)
- **Study method.**
  The dose of drug prescribed to the patient will be detailed in the patient's clinical couse (at the discretion of each physician) and this information will be added to the computerized database. The dose ranges usually used at the beginning of the introduction of the drug are detailed in section 3.2.3.

7.7.3. Vitamin B12

- **Type of variable.**
  It is a continuous quantitative variable**.**
- **Reason of selection.**
  Also known as cobalamin, it is considered a possible confounding variable because this vitamin also participates in the metabolic pathway of L-DOPA. (49)
- **Study method.**
  Chemiluminescent analysis of intrinsic factor microparticles for the quantitative determination of vitamin B12 in human serum and plasma.
- **Trademark.** Abbott Laboratories – Alinity®.
- **Sample.**
  For serum specimen type: serum collection tubes or serum separator.
- **Transport.**
  The laboratories of the study's reference centers have the necessary equipment.
- **Storage.**
  - At room temperature the maximum time is 3 days and as special instructions the specimens can be stored with or without the clot, erythrocytes or separating gel.
  - At a temperature between 2-8º the maximum storage time is 7 days and the special indications are identical to the previous section.
  - If these days are exceeded, the special instructions must be removed and stored frozen at a temperature below -20º.
- **Reference values (RV).** Listed in *table 15*
- **Coefficient of Variation (CV) and Total Error (TE)**
  CV 4,5% i TE 8.77%

7.7.4. Folic acid

- **Type of variable.**
  It is a continuous quantitative variable**.**
- **Reason of selection.**
  Also known as vitamin B9, it is considered a possible confounding variable because this vitamin also participates in the metabolic pathway of L-DOPA. (49)
- **Study method.**
  Chemiluminescent microparticle immunoassay (CMIA) is used for the quantitative determination of folate in human serum, plasma and erythrocytes.
- **Trademark.** Abbott Laboratories – Alinity®.
- **Sample.**
  Serum collection tubes or serum separator.
- **Transport.** The laboratories of the study's reference centers have the necessary equipment.
- **Storage.**
  - At a temperature between 2-8º the maximum storage time is 7 days. As special instructions, they must be protected from light.

  - At a temperature ≤ to -10º the maximum storage time is 30 days. As special instructions, they must be protected from light. Avoid performing more than 3 cycles of freezing and thawing.
- **Reference values (RV).** Listed in *table 15*
- **Coefficient of Variation (CV) and Total Error (TE)**
  CV 5.3% i TE 1.84%

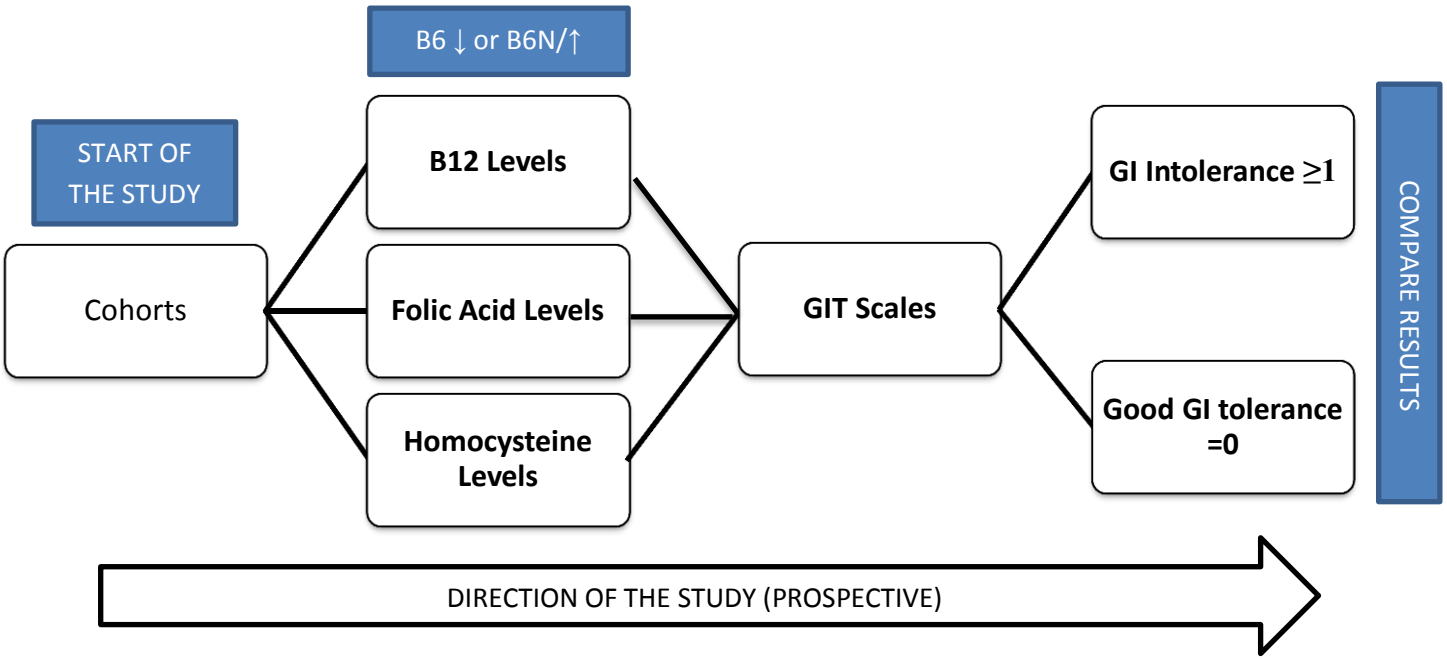
7.7.5. Homocysteine

- **Type of variable.**
  It is a continuous quantitative variable**.**
- **Reason of selection.**
  It is considered a possible confounding variable because this biomolecule also participates in the metabolic pathway of the L-DOPA.(49)
- **Study method.**
  Chemiluminescent microparticle immunoassay (CMIA).
- **Sample.**
  Serum or plasma with EDTA (ethylenediaminetetraacetic acid) or heparin.
- **Transport.**
  Some of the study's reference centers may need to transport these samples to an external laboratory for study.
- **Storage.**
  - Stable 4 days at 20-25ºC.
  - Stable 4 weeks if refrigerated between 2-10º.
  - Finally it is stable 4 years at -20º.
- **Reference values (RV).** Listed in *table 15*

Table 15 – B12, folic acid and homocysteine reference values

Reference values	
	Units of the international system
Vitamin B12	**In serum** 138-652 pmol/L
Folic Acid	**In serum** 3.1–20.5 nmol/L
Homocysteine	**In serum** Pregnant: < 10 umol/l Children < 15 years: < 10 umol/l Adults 15-65 years: < 15 umol/l Adults > 65 years: < 20 umol/l

** These reference values will allow us to classify the values of these covarites by levels: high levels (↑) /normal levels (N) / low levels (↓).*



7.8. Data collection

Being a prospective study the data necessary to initiate this study will be obtained as the study progresses.

At the **baseline visit** in which the patient is not yet medicated with L-DOPA the following points must be completed:

1. Provide the information sheet to the patient (Annex 1) which contains the necessary information about the study. The doctor will give the necessary time until the patient makes a decision and he will answer any doubts that may arise.
2. If the patient agrees to be part of the study, he/she must sign the informed consent (Annex 2) that the researcher will provide them.
3. At this point, patients will be provided with the **scale of gastrointestinal tolerance** that they must bring completed to the next follow-up visit (Annex 4).
4. Researchers will have to upload in the computerized database the doses of L-DOPA that they will prescribe to their patients.
5. The patient will be informed of the **possible adverse reactions** to the new drug and the **measures** to be followed in case of occurrence of gastrointestinal effects to the medication. Clinical expertise recommends that when gastrointestinal effects occur, the patient can take L-DOPA with meals or that the physician can prescribe *Motilium® 10mg/day* (3).
6. Finally, a request will also be made for these patients to perform a **blood test** that will analyze the parameters to be studied; B6, B12, folic acid and homocysteine. This analysis will allow obtaining the values of the independent variable and the covariables of the study. These analysis must be carried out as soon as possible.

The **follow-up visit** will take place **in 3 months** and the patient will be asked to provide us with:

- The **responded** gastrointestinal tolerance **scale.** The reference specialists will computerize the results and upload them to the database.
- Relevant clinical aspects to comment that have happened during the period between the basaline visit and this one.

The gastrointestinal tolerance scale to L-DOPA will allow the dependent variable to be assessed. The values presented in the questionnaire will be computerized to facilitate their subsequent work and will allow patients to be classified into:

- Gastrointestinal intolerance to LDOPA (TGI scale ≥ 1) or
- Good gastrointestinal tolerance to LDOPA (TGI scale = 0).

8. STATISTICAL ANALYSIS

The statistical analysis will be done with SPSS statistical software with the IBM® developer in its latest version available 26.0 and using the platform and programming language Java.

In all cases, a confidence interval (CI) of 95% will be assumed and a $p<0.05$ will be considered statistically significant.

8.1. Descriptive analysis.

A descriptive analysis of all baseline characteristics will be performed.

We will recategorize the independent variable, vitamin B6 levels, from having continuous quantitative characteristics to categorical characteristics, low or normal/high B6 levels. Therefore, an analysis will be carried out using percentages and proportions.

We will categorize the dependent variable on the presence or non-presence of gastrointestinal intolerance with L-DOPA. By having nominal dicothomic qualitative characteristics we will perform a descriptive analysis using percentages and proportions.

For the covariates, vitamin B12, folic acid and homocysteine, because they have also continuous quantitative characteristics, descriptive analyzes of each of them will be performed through means and standard deviation. Also, it will be stratified according to the intervention groups.

All analyses will be stratified by the sex and age of patients.

8.2. Bivariate inference

The different proportions between the onset of gastrointestinal intolerance or good tolerance to oral L-DOPA based on vitamin B6 levels will be evaluated using relative risk controlling the covariates and stratifying the results according to the sex and age of the patients.

Having both, the dependent and independent variable, categorical characteristics, we will use the Chi-Square test to compare the appearance of gastrointestinal intolerance between the two groups, exposed and non-exposed to the risk factor.

Student t-test will be used to analyze the association between B12, folic acid and homocysteine with the dependent variable.

These analyzes will be stratified by the covariables of the study.

8.3. Multivariate analysis

We will evaluate the influence of vitamin B6 levels (low or normal/high levels) on the onset of gastrointestinal intolerance (gastrointestinal tolerance scale score equal to 0 or ≥1) by logistic regression, adjusted for possible confounding variables.

9. ETHICAL ASPECTS

Lead researchers and collaborators guarantee that the study will be conducted in accordance with the ethical considerations and human rights collected in the *World Medical Association Declaration of Helsinki of Ethical Principles for Medical Research Involving Human Subjects, revised in the 64th General Assembly, in Fortaleza, Brazil, October 2013.*

This research protocol will be evaluated by the Clinical Research Ethics Committee *(CEIC)* of Hospital Universitari Doctor Josep Trueta and the other participating hospitals. The project will not begin under any circumstances until its approval.
The protocol will be carried out in accordance with the requirements of the *"Orden SAS/3470/2009 de 16 de diciembre sobre EPAs observacionales, de Estudios postautorización de tipo observacional".*

In order for a patient to be part of the study, it will be necessary that he/she is informed correctly, that they have enough time to assess the decision, as well as time to read the "Information for the patient" (Annex 1). All documents provided to the patient will be written in their vehicular language for their proper comprehension.

Subsequently they can freely sign the "Informed Consent" (Annex 2), a standardized document, which will be necessary to be included in the study, thus respecting the principle of patient autonomy and compliance with current regulations *"Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y de derechos y obligaciones en materia de información y documentación clínica*".

No compensation or financial expense will be obtained for their participation in the study. The patient's right not to participate in the study and to withdraw their consent at any time will be respected, and this will no cause any harm to their health care.

It will be guaranteed that all the information collected in this study will be confidential, guaranteeing the anonymity at all times of the participants who decide to be part of it in accordance with current regulations "*Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y garantía de los derechos digitales. BOE núm.294, de 6 de diciembre de 2018".*

In order to maintain the confidentiality of the data, a random identification number will be used instead of the name of the patients at the time of data analysis. The data will only be accessible to the researchers responsible for the study. No other external use other than the purpose of the investigation will be made.

The researchers declare that there are no conflicts of interest in this study.

10. STRENGHTS AND LIMITATIONS OF THE STUDY

- Our study is a prospective observational cohort study that has some disadvantages. Some important points are:
    - **High cost** and **longer realization** time.
    - Need to have a larger sample size.

- Being a prospective study we can calculate parameters such as the **incidence and those associated** with it. The relative risk will allow us to confirm or reject our initial hypothesis. In addition, using the incidence we can calculate other associated measures such as attributable risk, attributable fraction...

- The **strict inclusion criteria**, which only take into account the inclusion in the study of patients starting treatment with L-DOPA, greatly reduce the sample we can work on. The low sample size can make it difficult to extrapolate the results to the general population and therefore have a high internal validity but a low external validity. If the 'n' is increased, the differences due to chance will be reduced and the results will be more similar to the behavior of the general population.

- It is **not stipulated** which **initial dose of L-DOPA** use to start treating patients, being a decision of each physician which dose of L-DOPA will administer to each of his patients. Inspite of this, in section 3.2.3 the initial recommendations are defined. The dose of L-DOPA may be closely related to the study variables. (49) We will record the doses that each patient will receive from the study in the clinical course. This information can serve us for the future to raise new hypotheses.

- Regarding the **dependent variable**, Dra. Silvia Fàbregas i Puigtió, Clinical Chief of Digestology at the Hospital de Figueres, told us that to measure gastrointestinal tolerance to a drug there is currently **no validated table** for its routine study and its assessment is based on the consensus of experts.
    - The scale of gastrointestinal tolerance that will be given to us by the patients included in the study three months after the start of treatment with L-DOPA will present a **low memory bias**. The patient, if performed correctly, will record on the scale the presence of gastrointestinal intolerance at the time it appears during the period until the follow-up visit.
    - Using a scale in which the patient **subjectively evaluates the items** can lead to errors in their clinical interpretation, thus leading to an erroneous score, altering the results we intend to analyze. For this reason in Annex 4 we have included the description of the items to help patients in their correct interpretation.
    - Moreover other **pathologies may cause similar clinical**. Some of the exclusion criteria exclude those underlying pathologies that give these symptoms and also exclude those patients who are taking drugs that may have similar adverse reactions. During the study period, processes can appear that confuse the patient and at the same time us (such as

digestive processes, drugs ...). Therefore, we also recommend that patients consult the relevant specialist in case of doubt and state this in the follow-up visit to their reference neurologists.

- Regarding the **independent variable and the covariates**:
    - In order to compare the results obtained in all the hospitals and centers participating in the study, it will be used the same analytical reference values and the same methods of studies for B6, B12, folic acid and homocysteine indicated in section 7.6.2 and section 7.7.
    - The situation may arise that not all the participating reference centers have the necessary equipment to study these variables, which is why the need to transport blood samples to the centers with the suitable technology should be considered when the time comes.
    - There may be other covariates or confounding factors not considered that may influence the results.

- Being a **multicenter study**, the interpretation of the results may imply a certain degree of **interobserver variability**. To avoid this, professionals will be trained on how to collect data well (section 11). We have defined the parameters on the GIT scale (Annex 4) to minimize patient misinterpretations and their reference neurologist will be available to explain any doubts that may arise.

11. WORK PLAN AND CHRONOGRAM

The research team will coordinate, interpret and disseminate the results. Below we can see the sequence of activities that will take place.

- **Stage 1. Protocol development and its approval.**
  November 2020 – January 2021
  - **Study set-up.**
    - At the beginning of the study, the researchers will carry out a **bibliographic review** of the most relevant articles on the subject. They will formulate a first hypothesis of the study and its objectives.
    - Researchers will define the variables of the study and the methods for obtaining them.
    - The study coordinator will then draw up a preliminary protocol. Appropriate changes will be made before the final proposal of the study protocol is submitted at the request of the next item
  - **Ethical evaluation.**
    - The protocol will be reviewed by the *Comitè d'Ètica d'Investigació Clínica (*CEIC) *de l'Hospital Universitari Doctor Josep Trueta* and the CEICS of the other reference centers.
    - Once the appropriate modifications of the protocol that have been deemed necessary have been submitted, the study protocol will finally be approved.

- **Stage 2. Coordination and Organization.**
  February 2021
  - Once the protocol has been approved, we will contact all the reference centers that will participate in the study.
    - **First informative Meeting with Neurologists and Nurses.**
      It will have the purpose of informing and training all the other co-researchers who will participate in the study on the collection and interpretation of data, how to add the information obtained to the unified database, the criteria for selecting participants... Meetings will also be scheduled with the nursing teams of the participating hospitals to inform them of all the procedures that will be carried out during the study.
    - **First informative Meeting with Laboratory doctors.**
      We will inform them about the need to study pyridoxine, cobalamin, folic acid and homocysteine, the methods and equipment that will be used to measure them. The reference values that will be used for each of these biomolecules will also be provided. They must provide the results to the patient's medical history.
    - Informative meetings in order to coordinate the transport of samples that need to be analyzed in other reference centers that are equipped with the appropriate material.

- **Stage 3. Patient Inclusion and Data collection.**
  February 2021 – April 2022
    - Creation of a **unified database**, which will allow researchers to add information related to patients included in the study.
    - From this phase onwards, we will ask the co-researchers to begin the **inclusion** of patients in the study as long as they meet the selection criteria, thus defining our cohort.
    - In order to participate in the study, these patients must be provided with all the necessary information about the study (Annex 1) and subsequently must sign the informed consent (Annex 2).
    - **Blood tests** (Annex 3) will allow us to classify the selected cohort according to the presence or not of the risk factor. These blood samples will be studied in the reference centers with the specific methods discussed above. It is also proposed to transport these samples in case they do not have the necessary materials to be able to study it.
    - The next follow-up visit scheduled by their specialist will take place 3 months after the introduction of the L-DOPA. The patient must have filled the **scale of gastrointestinal tolerance** (Annex 4) that will be delivered at the basal visit.

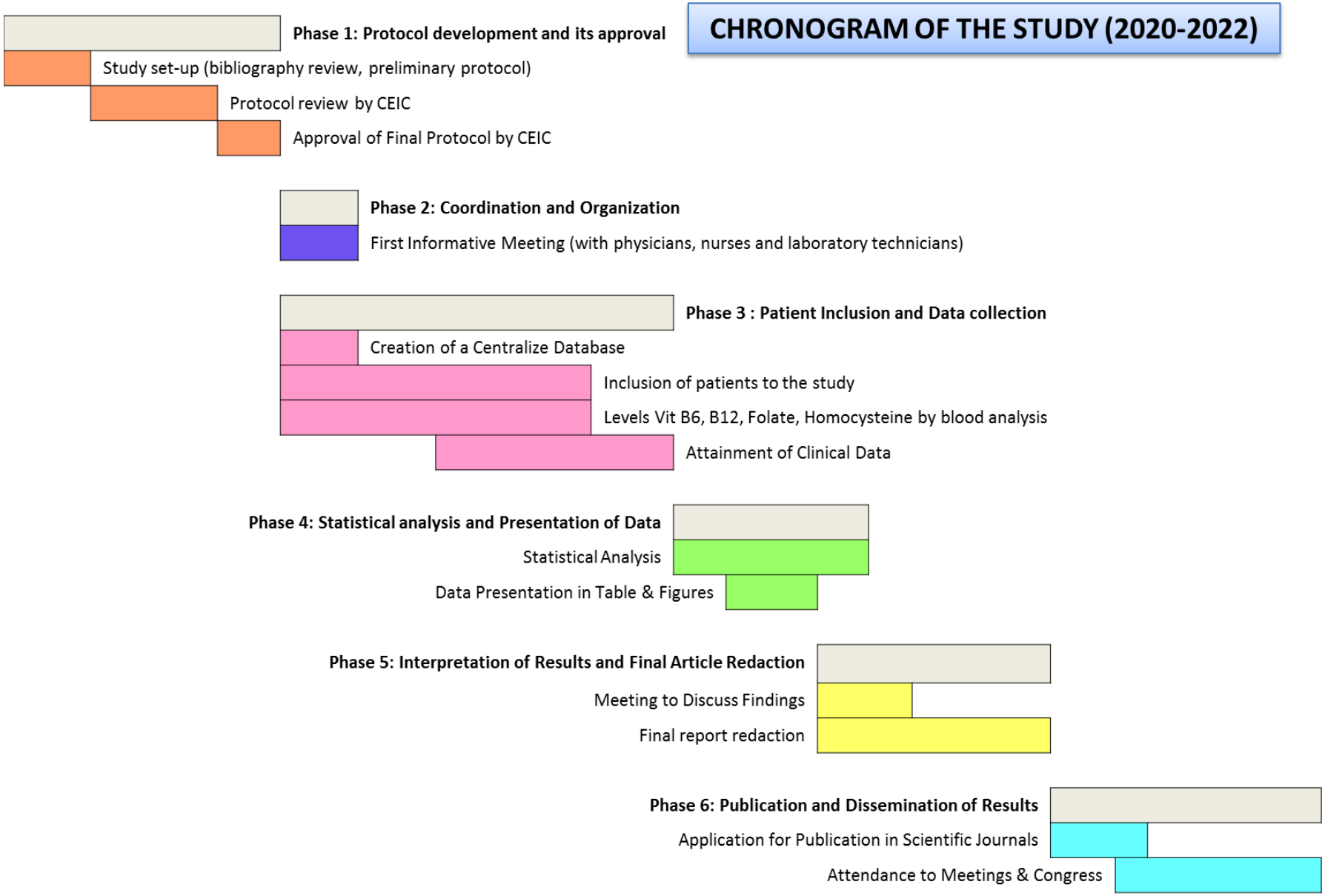
- **Stage 4. Statystical analysis and Presentation of Data.**
  May and June 2022
    - A qualified statistician will process the collected data and perform a statistical analysis. It will perform a descriptive analysis, a bivariate inference and a multivariate analysis.
    - The results obtained must be confirmed by a steering committee and a statistical committee.

- **Stage 5. Interpretation of Results and Final Article Redaction**
  June 2022 – August 2022
    - The statistical results obtained will be analyzed and discussed with the research team of the study to establish a definitive conclusion of the study.
    - Final writing of the manuscript stating the relevant findings of the study.

- **Stage 6. Publication and Dissemination of Results**
  September 2022 – Onwards
    - Submit the article for later publication in scientific journals.
    - Dissemination of results in conferences, workshops and meetings, among others.

CHRONOGRAM OF THE STUDY (2020-2022)

2020		2021			2022				
NOV 20	DEC 20	JAN 21	FEB 21	MARCH 21 – APRIL 22	MAY 22	JUNE 22	JULY 22	AUG 22	SEPTEMBER 22- ONWARDS



12. BUDGET

	Description	Quantity	Total	Cost
Personnel expenses				
Research coordinator	Person responsible for the study	1	20.000€/year x2 years	40.000€
Statistician	Person responsible for statistical analysis of data.	1	20€/h x 45h/weekly x 8 weeks	7.200€
Neurologists	People responsible for the control and monitoring of patients with PD included in the study.	-	-	0€
Nurses	People responsible for extracting the necessary blood samples from the patients included in the study.	-	-	0€
Laboratory technicians	People in charge of studying the blood samples taken from patients.	-	-	0€
Materials				
Blood tests [1]	Blood samples to study vitamin B6, vitamin B12, folic acid and homocysteine.	280	**Vitamin B6:** 31,05€/determination **Vitamin B12:** 3,11 €/determination **Folic acid:** 3,50€/determination **Homocysteine:** 7,50€/determination **Analytical cost 45,16€ per patient**	12.644,80€
Printing	Information sheet for the patient, informed consent, analytical	1680	**Cost per page 0,05€** Information for the	84€

[1] ***The blood test cost is determined based on the catalog of the ICS.***

	request and gastrointestinal tolerance scales.		patient: 3 sheets. Informed consent: 1 sheet. Analytical request: 1 sheet Gastrointestinal tolerance scales: 1 sheet. **Cost impressions /patient 0,3 € per patient**	
Publication expenses				
Scientific publication	Review, edition and format the article.	-	-	2000€
Dissemination [2] [3]	Attendance at national meetings and congresses.	-	-	800€
	Attendance at international congresses.	-	-	1300€
TOTALREQUESTED 64.028,80 €				

[2] ***The costs of attending the congresses are indicative and are broken down into registration (approximately 400-500€), transport (the cost will depend on the place where the congress is held) and finally meals and accomodation (depending on whether they are national or international about 200-400€).***

[3] ***Due to COVID-19 and the cancellation of most national and international medical conferences these costs despite being indicative may not be necessary.***

13. FEASIBILITY

This study is designed as a prospective observational study that will take place in several leading hospital institutions in Catalonia.

Due to its characteristics, an increase in costs in terms of patient follow-up will be avoided, as the follow-up visit at 3 months is regularly stipulated.

Health professionals (doctors and nurses) will not receive any additional financial compensation for being part of the study and therefore there will not be an increase in direct healthcare costs.

Only the project manager and the statistical analyst will receive economic remuneration.

The blood tests performed on patients will increase costs. It will not be necessary to train heath personnel again to perform this procedure.

There may be an increase in costs, if necessary, related to the need to renew medical equipment or materials (in case the reference centres do not have them) capable of performing the same technique of clinical analysis when studying the biomolecules involved in the study (B6, B12, folic acid and homocysteine). If it is finally decided to transport the blood samples to a single hospital (due to the lack of budget to obtain the relevant equipment), there is also the need to pay the transport company involved.

The study of the dependent variable, which is valued in scale format, will entail very small costs related to the printing of these, which later will be delivered to patients. Also, in relation to this aspect the information sheets and informed consents will entail added but very relative costs.

The duration of the study will be long enough to obtain a sufficiently representative sample and at the same time to assess the appearance of adverse reactions to the drug in question. Also, it will be enough time to assess the interindividual differences that the patients may comment on.

In addition, because the patient participation is relatively short (3 months from its inclusion), many patients will be prevented from being lost during the period between study inclusion, sampling, and delivery of gastrointestinal scales to L-DOPA.

We will hold an informative meeting with all the researchers and collaborators who are part of the study to explain and resolve all the necessary aspects, thus improving their efficiency and work. The necessary software will be provided to the health professional in order to work with the computer database that will be used later for statistical analysis.

To summarize, we consider this study presents all the appropriate requirements to be conducted considering the locations of the study, the economic cost and the amount of patients needed.

14. IMPACT ON HEALTH CARE SYSTEM

Parkinson's disease is a very prevalent pathology in our environment, with a high comorbidity associated. Dyskinesia, stiffness, tremors, instability... added to all the non-motor symptoms the patients may present, can cause great disability in these patients.

The pharmacological groups available today allow, with variability of response between patients, to improve the clinic the patients may present.

As we do not have drugs that stop or cure the disease, and it does not seem that this goal will be achieved in the coming years, PD progresses and this ends up with the necessity of having to increase the administered doses of the prescribed drugs, make changes in the pathways of drug administration, changes of pharmacological groups or combination of drugs to achieve better results. It should be remembered, as we had commented in the introduction and justification, that levodopa ends up being prescribed to most patients with PD because it remains the most effective.

If the hypothesis of the study is confirmed, we will be able to demonstrate that low pyridoxine levels are related to an increase in the onset of gastrointestinal intolerance in L-DOPA in patients with PD.

It should be remembered that due to the gastrointestinal adverse reactions to L-DOPA there is a worse adherence to treatment, and it causes us the unability to increase the dose when necessary because the patients will not tolorate the drug well. If we managed to improve the gastrointestinal tolerance to L-DOPA we would become more effective in treating patients, we would avoid adding more drugs in these patients such as dopaminergic agonists (which are more expensive than L-DOPA) and it would decrease the numbers of visits that these patients make with their specialists, which would mean a reduction inassociated costs. All of this without forgetting the most important aspect, we would improve the quality of life of these patients.

This conclusion may serve as a basis for new studies attempting to demonstrate experimentally whether by normalizing pyridoxine values before introducing L-DOPA in these patients we will be able to prevent the appearance of gastrointestinal intolerance to the drug; and also as a basis for new studies (asis already beginning to be done in our country) that try to demonstrate experimentally whether normalizing pyridoxine levels in patients with gastrointestinal intolerance to L-DOPA manage to decrease the clinic presented.

All these improvements could be achieved at a low cost, only by the price it would cost us to administer vitamin B6 in those patients who had their values decreased. It would be an affordable and efficient measure for the health system especially if we bear in mind that levodopa will continue to be in the medium term, and as it has been for more than 50 years ago, the drug of choice to treat this disease with high prevalence in our society.

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16. ANNEXES

16.1. Annex 1 – Information for the patient

FULL D'INFORMACIÓ AL PACIENT

Bon dia,

Agraïm la seva col·laboració en l'estudi que estem realitzant a tot Catalunya des del servei de Neurologia dels Hospitals Doctor Josep Trueta, de l'Hospital de Santa Caterina i de l'Hospital de Figueres i que ens ha de permetre millorar els coneixements que tenim actualment sobre el fàrmac, la levodopa, que a vostè recentment se li ha indicat com a tractament per a la Malaltia del Parkinson.

Amb aquest estudi els investigadors pretenem conèixer el paper que poden tenir els nivells de certes biomolècules en l'aparició de reaccions adverses gastrointestinals a la levodopa, el fàrmac d'elecció per a la majoria de pacients amb aquesta malaltia.

A partir d'aquest full informatiu pretenem respondre a les preguntes que vostè pugui presentar actualment i de cara al futur.

TÍTOL DE L'ESTUDI

Relació dels baixos nivells de piridoxina en l'aparició de intolerància gastrointestinal a la levodopa via oral en pacients amb Malaltia de Parkinson.

HOSPITALS INVOLUCRATS

Els hospitals involucrats en aquest estudi pertanyen a la demarcació de Catalunya i són:

- Província de Barcelona
    - Hospital Clínic i Provincial de Barcelona
    - Hospital de Bellvitge
    - Hospital Vall d'Hebron
    - Hospital de Sant Pau
    - Hospital del Mar
    - Hospital Trias i Pujol
    - Hospital Parc Taulí
    - Hospital General Sant Cugat
- Província de Girona
    - Hospital Dr. Josep Trueta
    - Hospital de Santa Caterina
    - Hospital de Figueres - Fundació Salut Empordà
- Província de Lleida
    - Hospital Arnau de Vilanova
    - Hospital Santa Maria de Lleida

- Província de Tarragona
    - Hospital Joan XXIII
    - Hospital Sant Joan de Reus

PARTICIPACIÓ

La participació a l'estudi és voluntària. Té l'opció de no participar o de retirar el seu consentiment en qualsevol moment i no suposarà cap perjudici per a la seva atenció sanitària.

No s'obtindrà cap compensació ni cap despesa econòmica per a la seva participació en l'estudi.

La seva participació en aquest estudi implica respondre unes escales de tolerància gastrointestinal que li proporcionaran i fer un anàlisi de sang. El seu metge de referència li proporcionarà el material necessari i la data o dates necessàries per realitzar l'extracció de sang.

Vostè com a participant té dret a ser informat dels resultats de la investigació que formarà part.

OBJECTIUS DE L'ESTUDI

Aquest estudi pretén estudiar si existeix una associació entre els nivells de la vitamina B6 (i altres biomolècules, vitamina B12, àcid fòlic i homocisteïna) en l'aparició de reaccions adverses gastrointestinals a la levodopa via oral.

Amb les dades que els metges i metgesses obtinguin a partir de les escales que vostè respondrà i dels valors obtinguts a l'analítica de sang que se li realitzarà s'estudiarà aquesta relació i la seva rellevància.

DESCRIPCIÓ DE L'ESTUDI

Aquest estudi té una durada aproximada de dos anys. L'entrega de les escales de tolerància gastrointestinal i l'analítica de sang es realitzarà un cop el pacient hagi signat el consentiment informat.

Aquest estudi comporta el mateix tipus de seguiment habitual que vostè ha realitzat fins ara per la Malaltia de Parkinson.

Un cop recollides totes les dades aquestes es descriuran i s'analitzaran estadísticament per obtenir-ne uns resultats. L'equip d'investigadors estudiarà aquests resultats per assolir unes conclusions respecte l'objectiu inicial de l'estudi i la seva rellevància futura en el sistema de salut.

BENEFICISI I RISCOS DE L'ESTUDI

Els riscos associats de l'estudi estan relacionats amb l'analítica de sang que se li realitzarà tal i com s'ha comentat prèviament i són:

- Sagnat excessiu.
- Desmai o sensació de mareig.
- Hematoma (acumulació de sang sota de la pell).
- Puncions múltiples per localitzar les venes,
- Infecció
- Dolor

Els beneficis que pot implicar aquest estudi representa un possible canvi a mig terme a l'hora de tractar les reaccions adverses dels pacients a la levodopa a via oral amb la conseqüent millora de la qualitat de vida.

CONFIDENCIALITAT DE DADES

Per al present estudi es prendran les mesures necessàries per garantir la confidencialitat de les seves dades, per utilitzar les seves dades únicament en aquest estudi i només amb fins d'investigació i que no en tinguin accés terceres persones en compliment de la normativa vigent (*Llei Orgànica 3/2018, de 5 de desembre, de Protecció de Dades Personals i Garanties dels Drets Digitals).* Les dades recollides seran gestionades de forma anònima.

REVISIÓ DE L'ESTUDI

Aquest estudi serà revisat pels Comitès d'Ètica d'Investigació Clínica dels hospitals de referència de l'estudi els quals garantiran que l'estudi compleixi la normativa actual i els protocols de d'ètica i bona praxis clínica.

MÉS INFORMACIÓ

En cas de continuar tenint dubtes a resoldre no dubti en contactar amb el seu neuròleg o neuròloga de referència.

En nom de la Unitat de Trastorns del Moviment dels Serveis de Neurologia de Catalunya;

Li agraïm la seva participació

16.2. Annex 2 – Informed consent

CONSENTIMENT INFORMAT

TÍTOL DE L'ESTUDI: Relació dels baixos nivells de piridoxina en l'aparició de intolerància gastrointestinal a la levodopa via oral en pacients amb Malaltia de Parkinson.

Jo, El Sr / La Sra ..... amb DNI .....

Afirmo que:

- He rebut i llegit el full informatiu que se m'ha lliurat.
- He pogut fer totes les preguntes necessàries respecte l'estudi i han estat respostes de manera satisfactòria.
- He rebut suficient informació sobre les característiques i objectius de l'estudi, els possibles riscos i la importància de la meva contribució per l'avanç de la medicina.
- He estat informat per l'investigador ..... de les implicacions i la finalitat de l'estudi i declaro que:
    - Comprenc que la meva participació és voluntària.
    - Comprenc que es respectarà la confidencialitat de les meves dades.
    - Comprenc que puc retirar-me de l'estudi en quan vulgui sense haver de donar explicacions i sense afectar la meva assistència sanitària.
    - Presto lliurement la meva conformitat per a participar a l'estudi.

Consento expressament a participar en l'estudi i entenc que la meva participació permet expressament el tractament de les meves dades personals i de salut, i manifesto que les dades facilitades per l'estudi són certes.

A dia ..... de ..... del .....

Signatura del participant Signatura de l'investigador

16.3. Annex 3 – Laboratory extraction

Institut Català de la Salut

Nº petició	

EXTRACCIÓ DE LABORATORI		
HOSPITAL: Porta d'Urgències		
Pàgina 1	1	1

Nom		Sexe		Nº història	
Data de naixement		Edat			
Nº Seguretat social				Data de registre	
Metge				Data d'extracció	
Origen				Centre d'extracció	
Sospita diagnòstica					
Observacions				Nº petició	
Tractament					

Tècniques sol·licitades

MOSTRES REQUERIDES	
Tubs de sèrum	1

PROVES SOL·LICITADES	TIPUS

Vitamina B6
Vitamina B12
Àcid fòlic
Homocisteïna

16.4. Annex 4 - Data collection sheet

FULL DE RECOLLIDA DE DADES

NOMS I COGNOMS: _____

DATA: _____ / _____ / _____

**Marqui amb una X la casella "Sí" en cas de presència dels símptomes o marqui la casella "No" en cas de no presència dels símptomes de l'escala en els últims tres mesos.*
***En cas de dubte consulti en el full on hi trobarà la descripció de cadascun dels ítems.*

Escala de tolerància gastrointestinal		
- **Nàusees**	Sí ☐	No ☐
- **Vòmits**	Sí ☐	No ☐
- **Dispèpsia**	Sí ☐	No ☐
- **Dolor abdominal i molèsties digestives**	Sí ☐	No ☐

Basis en aquestes definicions en cas de dubte o no coneixement del terme en qüestió

Nàusees	*Desig fort i desagradable de vomitar*
Vòmits	*Expulsió violenta per la boca de continguts estomacals*
Dispèpsia	*Dolor o molèstia a la zona alta de l'abdomen*
Dolor abdominal i molèsties digestives	*Sensació desagradable, com dolor o malestar, que pot sentir el pacient com les molèsties provocades per la ingestió d'aliments, el trànsit d'aquests o els moviments intestinals.*

Puntuació: Sí=1 punt // No =0 punts
Puntuació final màxima 8 // Puntuació final mínima 0

PUNTUACIÓ TOTAL: ____