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TITLE: A Multicenter Randomized Controlled Trial comparing the Efficacy of Transanal Irrigation versus Standard Bowel Care in patients with Parkinson Disease and severe constipation. Impact on Gut Microbiome and Motor Disorders. TAI-PD Study.

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A Multicenter Randomized Controlled Trial comparing the Efficacy of Transanal Irrigation versus Standard Bowel Care in patients with Parkinson Disease and severe constipation. Impact on Gut Microbiome and Motor Disorders. TAI-PD Study.

Spontaneous, non-sponsored study

Coordinator center: Azienda Ospedaliero-Universitaria Ferrara
Principal Investigators:
-Ascanelli Simona, Unit of Mini-invasive and robotic surgery of digestive tract-**University Hospital Ferrara, Italy**
CoPI: Sensi Mariachiara, Gozzi Andrea, Mechelli Alessandro-Neurology Unit-**University Hospital Ferrara, Italy**

Participating centers

1) Piscaglia Maria Grazia, Bloise Maria Carmela, Guerra Federica, Di Lorenzo Petia, Bombardini Cristina, Federico Zanzi-**ASL Romagna- Ravenna**
2) Giblino Giulia, Sambri Vittorio, Fabbri Carlo, Devergori Emiliano, Carlini Eleonora, Dirani Giorgio -**ASL Romagna-Forlì-Cesena**
3) Negrotti Anna, Vincenzi Francesca, Petraglia Federica, Rossini Matteo -**Ospedale Maggiore di Parma**
4) Pacillo Francesca, Salvemini Carlo-**ASL di Parma Ospedale di Fidenza**
5) Pelizzari Laura, Magnifico Fabiola-**ASL di Piacenza e Unità Spinale Ospedale Fiorenzuola D'Arda**
6) Morganti Lucia, Guttman Susanna, Frusciante Roberto**-San Marino Istituto per la sicurezza sociale**
7) Palumbo Pasquale, Kiferle Roberto, Terenzi Federica, Giani Jacopo, Del Gamba Claudia-**ASL Centro Prato**
8) Clementi Ilaria**- Policlinico Umberto I Roma**
9) Dialti Valeria, Zucchi Elena-**Presidio Ospedaliero Istituto di Medicina Fisica e Riabilitazione "Gervasutta", Azienda Sanitaria Friuli Centrale**
10) Manassero Alberto**-Azienda Ospedaliera Universitaria Città della Salute e della scienza Torino Ospedale CTO**

Background

Constipation represents a highly prevalent symptom in Parkinson Disease (PD) with estimates ranging from 20 to 80 percent and may be present as early as the prodromal phase, many years before the onset of motor symptoms, however, manifesting to varying degrees in all stages of the disease [1]. Constipation, difficulty evacuating stool, and fecal incontinence in PD are symptoms of the complex condition called neurologic bowel disease (NBD) due to injury to nerves involved in the control of gastrointestinal (GI) function as occurring in other neurological diseases (spinal cord injury (SCI), multiple sclerosis (MS), spina bifida, and cerebral palsy) [2].

The brain-intestinal-enteric axis is thought to play a key role in gastrointestinal motility through interactions between the central and enteric nervous systems. The causes of constipation in PD are partially known but this symptom seems to correlate with reduced neuronal density in the myenteric plexus and increased alpha synuclein, a biomarker of neurodegeneration in PD.

Alteration in the composition of the gut microbiota (GM) is indeed considered crucial in the pathogenesis of PD, and it has been emphasized in several studies over the past two decades [3-5] how it plays a primary role in the aggregation and pathological accumulation of α-synuclein in the enteric plexus and its subsequent dissemination to the brain. Recent meta-analyses have confirmed that the GM of PD patients is dysbiotic, with an overabundance of taxa associated with impaired barrier function and increased gut inflammation compared to healthy controls [6]. GM is known to influence colonic motility through the production of short-chain fatty acids (SCFAs) and other metabolites, potentially contributing to constipation [7]; however, the exact proportion of PD constipation cases that can be directly attributed to dysbiosis remains unclear and varies across studies. In addition, GM changes over the PD disease and is influenced by a variety of endogenous and exogenous factors, including dietary changes (such as the typical reduction in protein intake) and the use of PD and non-PD medications, which increase the severity of the gastrointestinal picture [8].

Levodopa absorption appears to be affected by intestinal transit and dysbiosis present in patients with PD. Slowed intestinal transit contributes to delaying, reducing, or even blocking the absorption of oral levodopa, potentially delaying or losing its effect. Moreover, GM has been shown to influence the bioavailability of drugs such as levodopa [9-11] and to contribute to the appearance of dopaminergic medication side effects by producing a deaminated levodopa metabolite, which inhibits ileal motility [12], perpetuating this vicious cycle.

Mechanistically, constipation in Parkinson's disease can be classified into:

- Constipation by slowed transit: due to prolonged intestinal transit time that is manifested by decreased frequency of evacuations (defecation every 4 to 5 days), bloating and abdominal pain;
- Constipation from obstructed defecation: difficulty in expelling stool is caused by incoordination of the pelvic floor muscles (failure to relax or paradoxical contraction of the anal sphincter) and is manifested by significant straining upon evacuation, often with incomplete emptying and a tendency to form fecalomas [13].

The management of constipation in PD is very important: incorrect management of constipation in PD leads to bowel obstruction and impaired therapeutic response to PD drugs due to defective absorption.

Traditional bowel management (SBC: standard bowel care) consists of increasing fiber and liquid intake with diet, abdominal massage, and the use of laxatives and evacuative suppositories or enemas. Administration of different types of probiotics and prebiotics was more effective than placebo. Probiotics appear to increase the frequency of evacuations. Some available pharmacological treatments are considered effective for the management of constipation in PD patients [14]. Polyethylene glycol, lubiprostone, prucalopride, mosapride and linaclotide have been

shown to improve the frequency of evacuations in patients with PD. Botulinum neurotoxin is also suggested for the management of constipation associated with PD.
For the treatment of constipation recently, retrograde transanal irrigation (TAI) of the colon has also been proposed in some neurological diseases to facilitate stool evacuation by introducing water into the colon through the anus [15,16]. By regularly emptying the bowel in this way, TAI aims to help restore control of bowel function and allow people to choose the time and place of evacuation, preventing bowel obstructions and fecal impaction, and improving abdominal pain and bloating.
The efficacy and safety of TAI has already been widely demonstrated in people with neurological bowel caused by a wide variety of causes, particularly in people with paraplegia, spina bifida, and multiple sclerosis [15,16].
To date, there are no studies in the literature examining the efficacy of TAI on constipation in patients with PD, and any effects also on motor symptoms and gut microbiota. To evaluate these aspects, a pilot study (confidential results, not published yet) was conducted at the Neurology Unit and General Surgery Unit of the University Hospital of Ferrara (Ferrara, Italy) with the collaboration of the Department of Pharmacy and Biotechnology, University of Bologna (Bologna, Italy). The aim of the study was to evaluate the safety and efficacy of TAI in PD constipation and secondly to assess GM changes after TAI treatment, motor and non-motor symptoms, PD medications, and quality of life. All PD patients referred to the Movement Disorders Outpatient Department of Ferrara University Hospital were consecutively evaluated between July 2022 and July 2023. In the TAI+ population (12 patients) scores for constipation and quality of life improved statistically significantly at 6 and 12-month follow-up, while outcomes of motor and non-motor symptoms remained unchanged. Instead, quality of life examined through PDQ-39 questionnaire achieved a statistically significant improvement at 12 months ($p<0.001$). Also, VAS improved significantly at 6 months after TAI use ($p<0.001$) and remained high until the end of follow-up. In the no-TAI group (14 patients), neurological and proctological assessments remained stable during one year of follow-up. No patients modified dopaminergic therapy in either group.
Of the 26 patients participating in the study, 8/14 no-TAI patients and 11/12 TAI+ patients provided faecal samples for GM analysis at baseline (T0). Nine/12 TAI+ patients also provided a faecal sample after 6 months of TAI use (T1). No significant differences in alpha diversity were found between groups at baseline or after TAI use in the TAI+ group. Taxonomically, the no-TAI and TAI+ groups showed no differences at baseline, but some changes were observed in the TAI+ group at T1. In particular, TAI use resulted in an increased relative abundance of the family Erysipelotrichaceae ($p=0.0045$) and tended to increase the proportions of Clostridia UCG-014 ($p=0.155$). It also tended to decrease the proportions of the phylum Bacteroidota ($p=0.128$) and the genera Faecalibacterium ($p=0.123$) and [Eubacterium] eligens group ($p=0.074$).
This pilot study showed that TAI is a safe and user-friendly option for patients or caregivers, with excellent results in terms of improved quality of life and bowel management. Moreover, TAI probably induces changes in GM composition, some of which may promote gut homeostasis. The limitations of this study were mainly related to the small sample size and the short follow-up, which did not allow more consistent data on GM and its temporal dynamics nor to draw a correlation between PD phenotypes and disease severity.
Based on these limitations, we are proposing a multicenter controlled study, with a larger number of patients, focusing on motor and non-motor symptoms after TAI use, with long-term follow-up.

Study Objectives

The hypothesis of this multicenter randomized controlled trial is that TAI is superior to Standard Bowel Care (SBC) for intestinal management with impact on gut microbiota and motor symptoms in PD patients suffering from NBD in the long-term.

The primary objective of the study is to evaluate the efficacy of TAI compared to SBC in the treatment of constipation related to Parkinson's Disease, as measured by the change in Cleveland Clinic (Wexner) constipation score [17] in the short (6 months) and long term (24 months).

Secondary objectives are:
-to evaluate change in the perception of impact of bowel symptoms on QoL after 6 months and 24 months of TAI treatment compared to SBC;
- to evaluate change in NBD symptoms after 6 and 24 months of TAI use compared to SBC;
-to evaluate change in fecal incontinence symptoms after 6 and 24 months of TAI use compared to SBC);
-to evaluate patient satisfaction about the treatment and intestinal management after 6 and 24 months of TAI use compared to SBC;
- to evaluate study therapy adherence after 6 and 24 months of treatment;
-to evaluate the association between GM composition in PD patients using TAI compared to controls in the short and long term (6 months and 24 months);
-to evaluate the association between motor and non-motor symptoms in the two groups and correlation with dopaminergic therapy in the short and long term (6 and 24 months);
- To evaluate the safety of TAI and SBC by assessment of adverse events, non-serious and serious, and TAI device deficiencies occurring during the study period.

Study design
We propose a randomized parallel trial (24-month duration) involving the treatment centers for Italian Parkinson's disease. This study is designed as a randomized, superiority, controlled, interventional, prospective, multicenter, post-market study of TAI (with Qufora Flow device, IrriSedo, Allerød, Denmark) versus SBC, conducted in a population of 200 patients with PD and confirmed NBD. Qufora device for TAI is routinely used for the management of intestinal symptoms in neurological diseases (NBD) and is prescribable with the SSN by the medical specialist [15,16].
The study will contain 3 scheduled on-site visits (Baseline, 6 months and 24 months after Baseline) and 2 follow-up (FU) interviews by telephone after 1 month and 12 months.
With a planned 12-month recruitment period, the total duration of the study is estimated to be approximately 3 years. Additional sites and a prolonged recruitment period may be needed in case the recruitment rate of subjects has been underestimated. The minimum number of subjects to be randomized for each site is 8, and no site may randomize more than 40 subjects.
Potential subjects will be asked to sign the Informed Consent Form (ICF) and will thereafter be assessed for eligibility. Study subjects fulfilling the eligibility criteria will be considered eligible for further study participation. An eligible subject will be randomized 1:1 into either the TAI arm or the SBC arm.
After randomization, the TAI arm will be treated with Qufora Flow and the subject will be trained in device use. SBC arm will include the same close contact with investigator or delegate as for the TAI arm including lifestyle advice and recommendations for laxatives agents.

Study setting
Neurology and gastroenterology/surgical outpatient clinics in public and university hospitals participating in the study.

Data will be collected in a shared CRF on an online platform.

Inclusion criteria

For inclusion in the study, subjects must fulfil all of the following criteria:

1. Provision of informed consent.
2. Female or male aged 18 years or above.
3. Established diagnosis of PD according to MDS criteria [18].
4. Patients with bowel symptoms post-dating and related to a diagnosis of PD.
5. PD patients suffering from constipation defined by Cleveland Clinic (Wexner) constipation score [17] ≥10 confirmed at Baseline.
6. Only TAI treatment naïve patient (not having previously used any particular TAI system).
7. Judged eligible for TAI as per standardized treatment pathway [15].
8. Able to read, write and understand information given to them regarding the study.

Exclusion Criteria

Any of the following is regarded as a criterion for exclusion from the study:

-Any confirmed or suspected diagnosis of anal or colorectal stenosis, active inflammatory bowel disease, acute diverticulitis, severe diverticulosis, colorectal cancer, ischemic colitis, history of life-threatening autonomic dysreflexia, bleeding disorders, unspecified peri-anal conditions.

-Other significant neurological diseases (defined as all neurological diseases except for minor functional neurological syndromes or non-PD related neuro complications).

-Opioid consumption ≤24 hours prior enrolment.

-Taking probiotics in the past 30 days.

-Antibiotic therapy in the past 3 weeks.

-Performed endoscopic polypectomy within 4 weeks prior enrolment.

-Ongoing, confirmed pregnancy or lactation.

-Any neuromodulation that can affect the pelvic organ function.

-Current treatment of prokinetics.

-Any other condition, as judged by the investigator, might make follow-up or investigations inappropriate.

Intervention

PD patients with severe disease-related constipation (with Cleveland Clinic (Wexner) score ≥10 [17]) screened at the Movement Disorders Neurology Outpatient Clinic (Neurology Unit) will be referred to the proctology outpatient clinic of the Unit of Minimally Invasive and Robotic Digestive Surgery for clinical evaluation, inclusion in the study, and randomization to TAI or SBC treatment.

Transanal irrigation (TAI)

TAI will be performed with the use of Qufora flow device (IrriSedo, Allerød, Denmark), following the current practice and guidance [19,20]. Qufora device for TAI is routinely used for the management of intestinal symptoms in neurological diseases (NBD) and is prescribable with the SSN by the medical specialist. Qufora Flow is a medical device for performing TAI, a treatment that instills water up into the lower part of the colon (through a hydrophilic catheter or cone), so the peristaltic muscles in the bowel can be trigged and start to evacuate the colon and rectum.

The water is introduced into the rectum by a manual propulsion system with the patient sitting on the toilet; the amount of irrigated water is 400 cc, and each patient or care giver will undergo specific training by dedicated staff. The duration of every TAI session is about 20 minutes.

It is very important that patients do bowel cleansing before the first TAI session, given the frequency of fecal impaction in PD patients that would prevent the intestinal uptake of water.
During the training period and during the first month of using the TAI, patients or care givers will be contacted by telephone for any doubts or difficulties, and training will be repeated if necessary.
TAI will be performed by PD patients or care givers every other day.
The efficacy and safety of TAI has already been widely demonstrated in people with neurological bowel caused by a wide variety of causes, particularly in people with paraplegia, spina bifida, and multiple sclerosis.

Infungibility features of the Qufora Flow product for TAI:

Qufora Flow is the only device that can be used for constant flow water introduction by gravity than by pulsed pressure. Gravity pressure allows a more controlled and natural water insertion that facilitates acceptance of the procedure and limits possible catheter ejection reactions that may affect patient compliance. Pulsed pressure allows variable pressure water insertion at regular intervals that simulate natural intestinal peristalsis.
Qufora Flow has a simplicity of assembly (only connection of the tube to the catheter) and use (one-handed handling) that facilitates the acquisition of full patient autonomy to the practice of irrigation even in patients with reduced cognitive abilities. In fact, the product's unique ergonometry ensures one-handed operation of the pump and flowmeter as well as fine-tuning of the flow of water introduced into the bowel. The unique cone catheter equipped with 5 holes (1 apical and 4 lateral) allows the creation of a vortex of water that promotes its ascent into the colon and thus the subsequent redescent of stool.

Standard Bowel Care (SBC)
SBC is defined as the best supportive bowel care without using irrigation. Bowel care will be scheduled at least every 2nd day, at the same time of the day and after ingestion of food and liquid to develop a habitual, predictable response and to take advantage of the gastro-colic response. Diet, fluids, abdominal massage, regular physical activity and medications will be used if non-interventional treatment is insufficient.
SBC protocol includes:
1) Fiber intake 25 g/day, at least 50% soluble → Bristol Stool Form (BSF) goal 3-5
2) Fluid intake Minimum 1.5 l/day
3) Abdominal massage Using the palm of the hand or a tennis ball or similar, pressure is applied and released firmly but gently clockwise around the abdomen, starting from the right lower quadrant.
4) Laxatives (Macrogol, or polyethylene glycol, psyllium, sodium docusate, lactulose, bisacodyl) to be chosen according to the patient's characteristics
Macrogol and Polyethylene glycol act as osmotic agents. They are quite effective, and the dose can be adjusted within a wide range according to the patient’s need. In patients with PD they have proved to relieve constipation [21]. Psyllium is a bulking agent which might work for slow transit constipation. A bulking agent might also work in patients who are wheelchair bound. The use of a stool softener, i.e. sodium docusate, might make it easier for the stool to pass. The use of the osmotic agent Lactulose should be cautious in patients with very mild symptoms. In fact, given the likely presence of pan-gut dysmotility it is likely to cause or aggravate symptoms of bloating, due to fermentation and production of methane, as well as being ineffective. A stimulant laxative such as bisacodyl has been shown to be very effective in patients with chronic idiopathic constipation.

5) Rectal and digital stimulation: if there is no spontaneous evacuation, Bisacodyl or Glycerin suppositories or a micro-enema, e.g. Microlax 5-7.5 ml, act as rectal stimulants, having a very rapid onset of action.

Outcomes

Primary outcome: To evaluate the efficacy of TAI compared to SBC in the treatment of constipation related to Parkinson's Disease, the change in the Cleveland Clinic (Wexner) constipation score [17] in the short (6 months) and long term (24 months) will be measured.

Secondary outcomes are:
-to evaluate change in the perception of impact of bowel symptoms on QoL after 6 months and 24 months of TAI treatment compared to SBC (outcome variable: change in PACQoL score [22] between baseline and 6 and 24 months in the TAI arm, compared to the SBC arm);
- to evaluate change in NBD symptoms after 6 and 24 months of TAI use compared to SBC (outcome variable: change in NBD score [23] between baseline and 6 and 24 months in the TAI arm, compared to the SBC arm);
-to evaluate change in fecal incontinence symptoms after 6 and 24 months of TAI use compared to SBC (outcome variable: change in Fecal Incontinence Cleveland Clinic Index (FI-CCI) [24]);
-to evaluate patient satisfaction about the treatment and intestinal management after 6 and 24 months of TAI use compared to SBC (outcome variable: VAS at 6- and 24-months treatment, as measured by a single question in the two groups [25]);
- to evaluate study therapy adherence after 6 and 24 months of treatment (outcome variable: number of subjects using TAI or SBC at 6- and 24-months treatment, as measured by a single question);
-to evaluate association between GM composition in PD patients using TAI compared to controls in the short and long term (6 months and 24 months) (outcome variable: change of GM composition at 6- and 24-months treatment);
-to evaluate the association between motor and non-motor symptoms in the two groups and correlation with dopaminergic therapy in the short and long term (6 and 24 months) (Outcome variable: change in Hoehn and Yahr scale (H&Y) [26]; the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale Part III (MDS-UPDRS III) [18], Montreal Cognitive Assessment (MOCA) [27]; Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (I- IV) [28]; Movement Disorder Society Non-Motor-Rating Scale (NMSS) [29], SCOPA–AUT scale [30], Parkinson's Disease Questionnaire (PDQ-39) [31], LEDD (Levodopa Equivalent daily dose) [32] between baseline and 6 and 24 months in the TAI arm, compared to the SBC arm;
- To evaluate the safety of TAI and SBC by assessment of adverse events, non-serious and serious, and TAI device deficiencies occurring during the study period.

Data management
Database management and quality control
The global objective of the data management process is to ensure timely delivery of high-quality data in order to satisfy both Good Clinical Practice (GCP) requirements as well as the requisites of adequate statistical analysis and reporting. It comprises all steps of data handling such as acquisition, processing, and utilization of data.

We will collect all clinical data in a longitudinal database to facilitate research procedures. Patient data will be recorded via computerized support with restricted and controlled access. REDCap is a software created to manage rapid and structured data collection in the field of research. The database server with the stored data is located in the institutional hospital data center.
The EDC (Electronic Data Capture) will be developed to:

- Design, build and implement a database for data collection.
- Manage the quality of the data by configuring the system so that there are checks on the data entered (format, range, etc.)
- Create automatic and manual queries for study monitoring
- Harmonizing data provided by different sources
- Export the data collected in formats useful for statistical processing

The study foresees the use of data collection forms elaborated by the authors of the study, in order to minimize the compiler's commitment and make the data immediately available anonymously. Each authorized investigator will be given a personal password, which will allow them to enter and access data concerning their patients. Patient data will be entered anonymously. All data collected will be managed in a safe and secure environment. The system used for data management is validated and is compliant with FDA 21 CFR part 11. The data transmission will be encrypted with a secure socket layer (SSL) technology and all changes to data are logged with a computerized timestamp in an audit trail.
To round up the safety of the data, the database server with the stored data is located in a secure data center and protected by a firewall.
To ensure a patient's safety and integrity of the clinical data, monitoring will be performed by at least one person on-site, according to the ICH-GCP guideline (E6) and approved standard operating procedures. Particularly the study will be conducted in accordance with the Declaration of Helsinki and the requirements of all applicable local and international standards, e.g. data protection law. Written informed consent of all patients will be obtained prior to data collection.

Randomization

A permuted block randomization with a 1:1 allocation ratio will be implemented in order to ensure the balance across treatment groups. Each block consists of a specified number of randomly ordered treatment assignments. The randomization list will be managed through a REDCap randomization module.

Statistical aspects

Sample size calculation

We determined the sample size on the primary endpoint defined as the mean difference in the Cleveland Clinic (Wexner) constipation scale between treatment arms. A two-sided t-test has been considered for two independent samples according to the following parameters:

- A type I error probability of 0.05
- Overall power of 0.8

The literature evidenced that comparing TAI with CBM (Conservative Bowel Management or SBC) at the termination of the study, the mean (SD) scores were as follows: Cleveland Clinic constipation scoring system (range, 0-30, 30 = severe symptoms) was 10.3 versus 13.2 (SD=4.4), corresponding to a standardized effect size of 0.66 [33].
The achieved study size consists of 38 patients per study arm. Adjusting for a dropout of 20%, 94 patients will be included in the trial (47 per arm).

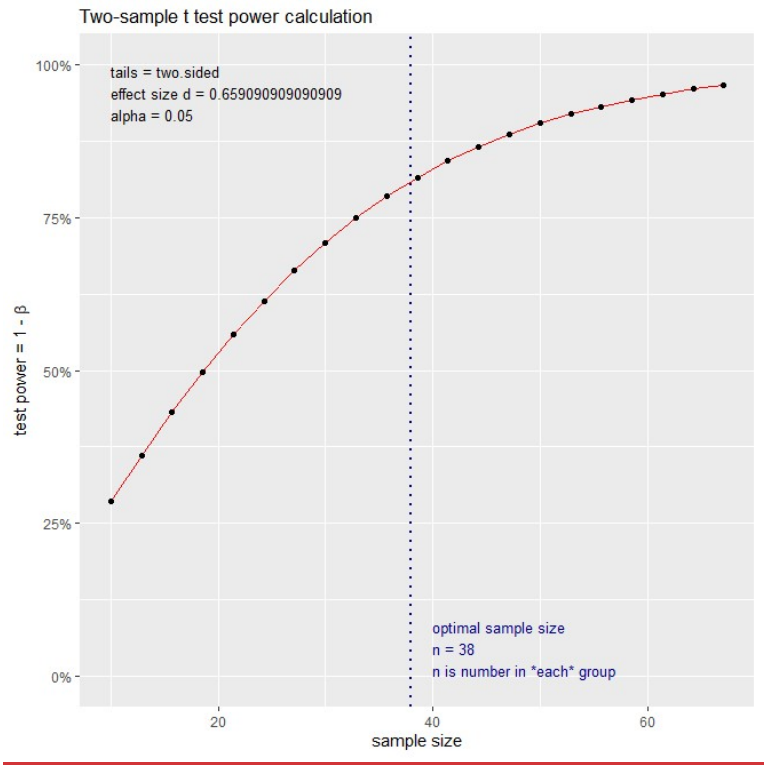


Figure 1 Statistical power according to sample size.

The computations have been performed using R 3.4.2 software and the package [34].

Statistical Analysis

Baseline data will be synthesized as median (I and III quartiles) for continuous variables and percentages (absolute numbers) for qualitative ones.

The primary efficacy analyses will consider mixed-effects models to assess the impact of TAI compared to SBC on the constipation scores among Parkinson's Disease patients. These models are adept at handling the complexities associated with repeated measures data, such as the correlation between observations taken from the same patient over time.

For binary outcomes, logistic regression models will be adapted to a mixed-model framework to evaluate changes in dichotomous outcomes across different time points. This will involve examining the likelihood of achieving a specific clinical threshold, such as significant improvement in bowel symptom scores.

Regarding the gut microbiota data, the statistical approach will focus on changes in microbial diversity and composition due to the interventions. This analysis will be conducted using multivariate techniques suited for ecological data, such as Principal Coordinates Analysis (PCoA) to visualize differences and similarities in microbial communities. The statistical significance of these differences will be tested using permutation tests based on pseudo-F ratios. Moreover, to assess the relative abundance of different microbial taxa, the team will employ generalized linear mixed models, providing a way to account for the repeated measures structure of microbiota data.

Adjustments for multiple comparisons will be made using the Benjamini-Hochberg procedure to control the false discovery rate, ensuring the reliability of the conclusions drawn about the microbiome's role in the treatment effects observed.

The primary outcome will be evaluated on the Intent-to-Treat population ITT.

A significance level of 0.05 will be considered to perform the final inference. Analyses will be conducted with R 3.4.2 [34].

Data collection

Participants will undergo a neurological and gastroenterological/surgical examination at baseline (T0) and 6 months (T1) and 24 months (T2) after enrollment.

	T0 - baseline	Phonecall (4 weeks)	T1 (6 months)	Phonecall 12 months	T2 (24 months)
Neurologic visit with LEDD calculation	X		X		X
Gastroenterological/Colorectal visit	X		X		X
UPDRS I-	X	x	X	X	X
H&Y	X	x	X	X	X
SCOPA AUT	X	x	X	X	X
NMS	X	x	X	X	X
PDQ 39	X	x	X	X	X
MOCA	X	x	X	X	X
TAI adherence	X	X	X	X	X
Cleveland Clinic (Wexner) Constipation score	X	X	X	X	X
PAC-QoL	X	X	X	X	X
NBD score	X	X	X	X	X
FI-CCI score	X	X	X	X	X
VAS Satisfaction (bowel habits)	X	X	X	X	X
GM analysis	X		X		X
Food diary	X		X		X

Visit 1 (T0): Screening and Baseline

Patients will undergo a clinical examination and several validated questionnaires on Parkinson's symptoms and bowel dysfunction after providing the informed consent.

During the first visit (T0) eligible PD patients suffering from constipation defined by Cleveland Clinic (Wexner) constipation score [17] $\geq$10) will be randomized to TAI treatment or SBC.

During the examination, information will be collected about illnesses and previous hospitalizations or surgeries, all medications taken, duration of constipation (particularly whether constipation was already present before PD diagnosis and for how long), and stool type (according to The Bristol Scale [35]).

Patients will also be tasked with completing a food diary for 2 weeks.

The Parkinson's disease-related questionnaires are as follows:

-Hoehn and Yahr scale (H&Y): scale commonly used to assess disease status [26];

- Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale Part I- IV (MDS-UPDRS): validated scale to assess motor symptoms in PD [26];

-Montreal Cognitive Assessment (MOCA): a brief, first-level assessment battery to assess cognitive impairments [27];

-Movement Disorder Society Non-Motor-Rating Scale (MDS-NMS): validated scale to assess non-motor symptoms in PD [28];

-SCOPE -AUT to assess dysautonomic symptoms [30];

-Parkinson's Disease Questionnaire (PDQ-39): validated questionnaire to assess health and well-being status in MP patients [31];

- diary of drug therapy used for MP and calculation of LEDD (Levodopa Equivalent daily dose) [32].

The bowel management-specific questionnaires will be:

- Cleveland Clinic (Wexner) constipation score: score from 0 to 30, assesses different aspects of defecation (frequency of defecation, difficulty, incompleteness, duration of defecation,

unsuccessful attempts, abdominal symptoms and duration of problem, regardless of laxative intake) [17];
-Patient Assessment of Constipation QoL questionnaire (PAC-QoL), a self-administered questionnaire divided into two sections: from B1-B6 questions assess the 'impact of altered bowel function in daily life while B7 measures patients' satisfaction with bowel management [22];
-Neurogenic Bowel Dysfunction Score (NBD): a 10-question questionnaire with a total score between 0 and 47, corresponding to the severity of bowel disorders present [23];
-Fecal Incontinence Cleveland Clinic Index (FI-CCI) to measure the level of fecal incontinence [24];
-Visual Analogue Scale (VAS), a scale from 0 to 10 that measures patients' satisfaction with bowel treatment and management [25].
All applicable data will be registered in the eCRF and the subject's medical record with relevant information about the visit will be updated.

Fecal sample for GM analysis: collection, storage, and analysis
Enrolled patients will be asked to bring a fecal sample (for GM analysis) before the start of therapy (TAI or SBC). The patient will be given guidance about how to collect stools for GM analysis. All centers will follow the same fecal specimen storage procedure. Feces will be stored in a freezer at -80°C. All the fecal samples will be sent in dry ice and analyzed by the Microbiology Laboratory of the Cesena Campus/Single Laboratory of the AUSL of Romagna, in Pievesestina, Cesena, Italy.
Management of the GM data will be carried out of Microbiology Laboratory of the Cesena Campus/Single Laboratory of the AUSL of Romagna, in Pievesestina, Cesena, Italy.
Quality and abundance data will be linked to the main clinical data via an anonymized alpha numeric unique ID record.

Telephone call after 1 month
This FU phone calls will be initiated by the investigator or delegate with the intention of identifying optimal performance and outcomes of the therapy. If there are specific problems reported by the subject, or there is inadequate efficacy of treatment, the investigator or delegate will advise tailoring of the regime. The subject's health status and adherence to the therapy (TAI or SBC) will be monitored, and any AEs/DDs that have occurred since last time and changes in medication(s) will be recorded.
Additional study specific phone call and/or visit except for those specified above, will be scheduled by the investigator or delegate only if needed.
All applicable data will be registered in the eCRF and the subject's medical record will be updated about the telephone contact.

Visit 2 (T1): Follow-up at 6 months
Patients will be reassessed at 6 months with the above-mentioned questionnaires. In addition, a new fecal sample will be collected. A food diary and medication diary will be collected again. Any adverse events will be reported.
Adherence to TAI treatment will also be checked at this visit.
All applicable data will be registered in the eCRF and the subject's medical record with relevant information about the visit will be updated.

Telephone call after 12 months
This FU phone calls will be conducted by the investigator or delegate with the intention of identifying optimal performance and outcomes of the therapy. If there are specific problems reported by the subject, or there is inadequate efficacy of treatment, the investigator or delegate

will advise tailoring of the regime. The subject's health status and adherence to the therapy (TAI or SBC) will be monitored, and any AEs/DDs that have occurred since last time and changes in medication(s) will be recorded.
Additional study specific phone call and/or visit except for those specified above, will be scheduled by the investigator or delegate only if needed.
All applicable data will be registered in the eCRF and the subject's medical record will be updated about the telephone contact.

Visit 3 (T2): Follow-up at 24 months
Patients will be re-assessed at 24 months with the above-mentioned questionnaires. In addition, a new fecal sample will be collected. A food diary and medication diary will be collected again. Any adverse events will be reported.
Adherence to TAI treatment will also be checked at this visit.
All applicable data will be registered in the eCRF, and the subject's medical record with relevant information about the visit will be updated.

Gut microbiota (GM) profiling
Microbial DNA will be extracted from feces using the repeated bead-beating plus column method [36] with a few minor adjustments as previously described [37]. The V3-V4 hypervariable regions of the 16S rRNA gene will be amplified using the 341F and 785R primers with Illumina adapter overhangs according to the Illumina protocol "16S Metagenomic Sequencing Library Preparation" (Illumina, San Diego, CA, USA). PCR products will be purified using a magnetic bead-based system (Agencourt AMPure XP; Beckman Coulter, Brea, CA, USA), followed by sample indexing by limited-cycle PCR using Nextera technology. The indexed libraries will be purified and pooled at an equimolar concentration of 4 nM. According to the manufacturer's instructions, the pool will be denatured and diluted to 5 pM before sequencing on an Illumina MiSeq platform using a 2 × 250 bp paired-end protocol. Sequencing reads will be deposited in the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA; BioProject: PRJNAXXX).
Raw sequences will be processed using PANDASeq [38] and QIIME 2 [39]. After filtering for length and quality, reads will be binned into amplicon sequence variants (ASVs) using DADA2 [40] and taxonomically classified using the VSEARCH algorithm [41] against the SILVA database (August 2020 release) [41]. Alpha diversity will be calculated using various metrics, such as the number of observed features, the Shannon index and Faith's phylogenetic diversity. Beta diversity will be calculated using UniFrac distances, which will be used to construct Principal Coordinates Analysis (PCoA) plots.

Training of Site personnel
Investigator and site personnel training will take place prior to subject enrollment to ensure that treatment with TAI and SBC will be done according to this protocol and the IFU (for Qufora Flow, Irrisedo), that complete, accurate and timely data are submitted, that protocol requirements are followed, and that any complications, and AEs are correctly reported and investigated, as appropriate. The site personnel will also be trained to use the eCRF system prior to first subject enrollment. The Principal Investigator shall ensure that appropriate training relevant to the study **is** given to all site personnel, and that any new information of relevance to the performance of this study is forwarded to the site personnel involved.

Data monitoring
The Principal Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data recorded in the eCRFs. Completed sections of eCRFs will be monitored on a regular basis.

Adverse events (AEs)
The safety measurement will be the onset, duration and frequency of AEs (anticipated and unanticipated), including determination of seriousness and causality. Only events, which are new after the consent has been signed or have increased in severity after the consent has been signed, will be recorded. All AEs will be recorded and reported, and all data required both to assess the safety and to comply with the EC and any other authority requirements will be collected.

Audits
The statistician will systematically and independently examine all study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported according to the CSP, ISO 14155 Good Clinical Practices and any applicable regulatory requirements.

Discontinuation
Subjects may be discontinued from study participation/treatment at any time. Specific reasons for discontinuing a subject from this study are:
– Voluntary discontinuation by the subject who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment.
– Safety reasons as judged by the investigator.
– Severe non-compliance to protocol as judged by the investigator, e.g. subject is not using the study treatment (TAI or SBC) that the subject has been allocated.
– Incorrect enrolment i.e. a subject does not to meet the required inclusion/exclusion criteria for the study.
– Subject lost to follow-up.
– Necessary to treat the subject with any of the medications listed as restrictions.

Ethical approval
Before subject enrolment and any study-related procedures are undertaken at a participating site approval of the study by the EC should be fulfilled.
The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki. Furthermore, the study will be performed in compliance with ISO 14155 and applicable local regulatory requirements.

Amendments to the protocol
In case of ongoing adherence of new centers an amendment will be requested from the ethics committee.

Informed consent (ICF)
The Principal Investigator or delegate at each site will:
– Obtain ICF from each subject before any study-related procedures are initiated. This includes the questionnaires and the collection and use of biological samples (feces) from participants

– Ensure each potential subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study (using non-technical, understandable language)

Disclosure

There is no financial or other conflict of interest by the researchers responsible for the trial and each participating center.

Data Protection

Patient Information Sheet will incorporate wording that complies with relevant data protection and privacy legislation. Pursuant to this wording, subjects will authorize the collection, use and disclosure of their study data by the Principal Investigator and by those subjects who need that information for the purposes of the study.

The principal Investigators Ascanelli Simona of "Azienda Ospedaliero-Universitaria di Ferrara", as Data Controller, will process your personal data only to the extent that they are indispensable in relation to the objective of the study, in compliance with the provisions of current legislation on the protection of personal data, including Regulation (EU) 2016/679 (General Regulation on the Protection of Personal Data), D. Lgs. June 30, 2003, No. 196 as amended (Personal Data Protection Code), and in accordance with the safeguards and any other applicable provisions of the "Garante per la protezione dei dati personali".

The personal data of the Interested Party and those belonging to particular categories, specifically those pertaining to genetic data and/or biological samples are collected and processed in order to carry out the above-mentioned scientific research, the characteristics and methods of which are described in detail in the document "Patient Information Sheet."

In order to participate in this research, the Interested Party will also be asked to consent to the Data Controller in question receiving the data collected and stored by the Azienda Ospedaliero-Universitaria di Ferrara in the context of normal clinical practice/as part of the above-mentioned project. The Data Controller will process said information and collect additional data concerning him/her exclusively for the purposes described herein and only if he/she consents to the research.

The data will be processed through paper and/or electronic means (in particular, the data will be recorded in a "data collection form", so-called "CRF" of paper and electronic nature").

Biological samples and genetic data will be retained until the end of the research, unless the Data Subject consents to retention for a longer period within the scope of the purposes. At the end of the research (scheduled for 5 years from the time of collection), the data and samples will be destroyed or anonymized by providing a final and irreversible deletion of the correspondence between the code used on the sample and the association of that code with the identity of the participant.

If the Respondent agrees to take part in this study, he/she is informed that-in addition to the above-mentioned subjects participating in the research-the data may be communicated to the relevant Regulatory Authorities, the Emilia centro wide area independent ethics committee (CE-AVEC), and the Italian health authorities who may examine all the health records of the Respondent collected during the study: the purpose of these verifications is to check that the research is conducted correctly and in accordance with the regulations in force, and the Respondent will not have the power to prevent such communications.

Special data and genetic data will not be disseminated. Research results may be subject to dissemination but, in that case, the Data Controller guarantees that they will be disseminated only in aggregate form or in a manner that does not make the data subjects identifiable even by indirect identification, including in the context of scientific publications, statistics and scientific conferences.

Data Access
All researchers participating in the trial will have access to the final trial dataset.

Dissemination
The results of the trial will be communicated to other health care professionals, Parkinson's disease associations and care-givers of Parkinson's patients through publications, conferences, and public events.

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