the epidemiology of infections with Clostridioides difficile and multidrug-resistant bacteria, and faecal microbiota transplantation as an intervention strategy

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Chapter 8

Safety and feasibility of faecal microbiota transplantation for Parkinson's disease patients: a protocol for a self-controlled interventional donor-FMT pilot study

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Abstract

Introduction

Several experimental studies suggest a role of gut microbiota in the pathophysiology of Parkinson's disease (PD) via the gut-brain axis. The gut microbiota can also influence the metabolism of levodopa, which is the mainstay of treatment of PD. Therefore, modifying the gut microbiota by faecal microbiota transplantation (FMT) could be a supportive treatment strategy.

Methods and analysis

We developed a study protocol for a single centre, prospective, self-controlled, interventional, safety and feasibility donor-FMT pilot study with randomization and double blinded allocation of donor faeces. The primary objectives are feasibility and safety of FMT in PD patients. Secondary objectives are to explore whether FMT leads to alterations of motor complications and PD symptoms in the short term, determine alterations in gut microbiota composition and donor-recipient microbiota similarities and their association with PD symptoms and motor complications, assess the ease of the study protocol and examine FMT-related adverse events in PD patients. The study population will consist of 16 idiopathic PD patients that use levodopa and experience motor complications. They will receive FMT with faeces from one of two selected healthy human donors and will be pretreated with vancomycin, bowel lavage, and domperidone. The FMT will be performed via a gastroscope. There will be seven follow-up moments during twelve months.

Ethics and dissemination

This study was approved by the Medical Ethical Committee Leiden Den Haag Delft (ref. P20.087). Study results will be disseminated through publication in peer-reviewed journals and international conferences.

Trial registration number

International Clinical Trial Registry Platform: NL9438.

Article summary

Strengths and limitations of this study

- Strict surveillance of (serious) adverse events.
- An autologous suspension for potential rescue treatment will be stored.
- Two different donors
- A broad range of clinical rating scales.
- Design includes a standard-of-care measurement for comparison of measured changes
- Multiple time points for gut microbiota analysis.
- No comparator arm with placebo

Introduction

Parkinson's disease (PD) is characterised by the degeneration of neurons and the presence of Lewy bodies and Lewy neuritis in the central nervous system (CNS), enteric nervous system (ENS) and peripheral autonomic nervous system. [1] The etiology and pathogenesis of PD is largely unknown, although a role for the aggregation of alpha-synuclein (αSyn) is generally acknowledged.[2]

GI symptoms are frequently observed in PD patients and often precede the onset of motor symptoms.[3,4] Alpha-synucleinopathy is present in the ENS and vagal nerves in an early phase of disease.[5-10] This led to the hypothesis that the disease may start in the gut.[5,11-14] The hypothesis is supported by studies suggesting that αSyn forms could be transported from the gut to the brain.[12-14] It is suggested that aggregation of αSyn in the brain and gut is a consequence of inflammation-induced oxidative stress.[15-17]

The gut microbiota and their metabolic products in PD patients differ from healthy individuals, with a more pro-inflammatory and less anti-inflammatory composition in PD.[18] Specific taxa appear to be associated with symptom severity[19] and gut bacterial tyrosine decarboxylases can metabolise levodopa to dopamine without being susceptible for carbidopa, which may alter the bioavailability of levodopa.[20,21]

It is hypothesised that interventions aimed at modifying the gut microbiota could influence PD symptoms severity and disease progression and/or improve levodopa absorption and efficacy, resulting in a decrease of levodopa-mediated motor complications. Faecal microbiota transplantation (FMT) could potentially restore the disturbed gut microbiota composition and metabolic activity of the microbiota.[22-24] FMT is an effective and safe treatment for multiple recurrent[25] and severe [26] *Clostridioides difficile* infections (CDI). Serious adverse events in this patient category have been described, but occur in only 0-5% of patients.[27-29] Currently, CDI is the only registered indication for FMT,[30-32] but preliminary data on FMT in several neurological disorders are becoming available.[33]

Since there are no available treatments to cure or slow down the progression of PD, the development of a new treatment strategy is crucial. A potential beneficial effect of FMT in PD is shown in several mouse studies.[34-36] Recently, one case report[37] and three case series (15, 11, and 6 patients)[38-40] have been published reporting the results of FMT in PD patients. Gut microbiota analysis was reported in only one case report and one case series (11 patients),[37,40] which showed significant changes in the gut microbiota. However, large variability of methods concerning pre-treatment, FMT administration route, follow-up and clinical evaluation exists. No results of randomised clinical trials on FMT in PD patients have been reported yet.

We report the protocol of a single centre, prospective, self-controlled, interventional safety and feasibility donor-FMT pilot study with randomization and double blinded allocation of donor faeces. The primary objective of the study is to demonstrate that FMT is feasible and safe in this patient group. We also hypothesise that FMT will lead to a decrease of motor complications, and improvement of PD symptoms, in the short term. Considering the scanty availability of data on FMT in this patient population, we have decided to focus on treatment safety without a control group. To correct for the known variability in occurrence and severity of both motor and gastrointestinal symptoms, we have introduced a 'standard-of-care evaluation' as comparator for these outcomes. In order to control for possible donor-related effects, faeces of two donors will be randomly assigned.

Methods and analysis

Objectives and study design

The study was designed in close collaboration with the Dutch Parkinson patients association, with participation of "patient researchers". The primary objectives are to assess feasibility and safety of FMT in PD patients. Secondary objectives are to explore whether FMT leads to alterations of motor complications (fluctuations or dyskinesias) and PD motor and non-motor symptoms (including constipation) in the short term, determine alterations in gut microbiota composition and donor-recipient microbiota similarities and their association with PD symptoms and motor complications, assess the ease of the study protocol and examine FMT-related adverse events (AEs) in PD patients.

The study is a single centre prospective self-controlled interventional safety and feasibility donor-FMT pilot study with randomization and double blinded allocation of donor faeces. Sixteen patients will be included. The follow-up period will be twelve months. The study site is Leiden University Medical Center. The 2013 SPIRIT checklist and the more detailed approved study protocol (version 4.2 January 2023) are shown in the Supplementary S6 and S3 files. Table 1 provides an overview of all study procedures. In Figure 1, an overview of the study design is shown.

Table 1. Schedule of study procedures.

	STUDY PERIOD								
VISIT	**V1**	**V2 (baseline)**	**V3 (standard-of-care)**	**V4 (treatment)**	**V5**	**Tel1**	**Tel2**	**V6**	**V7**
TIMEPOINT#	**$-t_1$**	**0**	**t_1**	**t_2**	**t_3**	**t_4**	**t_5**	**t_6**	**t_7**
ENROLMENT:									
Eligibility screen	X								
Informed consent	X								
Information on the study and on FMT	X								
Allocation		X							
ASSESSMENTS:									
Baseline questionnaire*		X							
Diary (3 days before visit)		X	X		X	X	X	X	X
Patient questionnaires**		X	X		X	X	X	X	X
MDS-UPDRS on medication***		X	X		X	X	X	X	X
Hoehn and Yahr		X	X		X			X	X
SENS-PD		X	X		X			X	X
MOCA		X	X		X			X	X
Registration of (S)AEs			X	X	X	X	X	X	X
Stool sample		X	X		X		X	X	X
Blood sample	X				X			X	

-t_1: screening visit: unspecified timepoint, t_1: 1 week after baseline (standard-of-care), t_2: FMT:1 week after standard of care, t_3: 1 week post-FMT, t_4: 2 weeks post-FMT, t_5: 6 weeks post-FMT, t_6: 3 months post-FMT, t_7: 12 months post-FMT *The baseline questionnaire includes questions on health status, disease-related variables and medication use (PD and non-PD). **Patient questionnaires are filled in by the participant prior to a visit/telephone appointment and include questions on health status, diet, medication use, constipation (Cleveland clinic constipation score[41] and ROME IV constipation criteria[42]), SENS-PD [43] Q10 (wearing off),[44] and MDS-UPDRS IB and II (and a study load questionnaire at V6).[45] *** MDS-UPDRS IA, III and IV (III not during telephone appointments).[45] Abbreviations: FMT: faecal microbiota transplantation, MDS-UPDRS: Movement Disorder Society Unified Parkinson's Disease Rating Scale,[45] MOCA: Montreal Cognitive Assessment,[46] (S)AEs: (serious) adverse events, SENS-PD: SEverity of Non-dopaminergic Symptoms in Parkinson's Disease,[43] Tel: telephone appointment, V: visit.

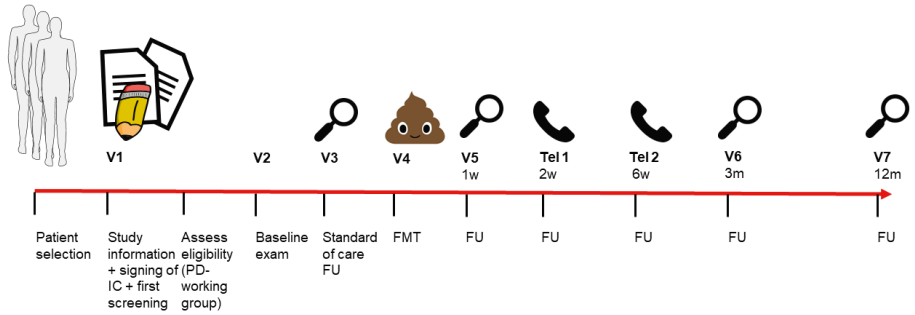

Figure 1. Graphical abstract of study design.

Abbreviations: FMT: faecal microbiota transplantation, FU: follow-up, IC: informed consent, m: months, PD: Parkinson's disease, Tel: telephone appointment, V: visit, w: week(s).

Patient selection and characteristics of study population

PD patients will be primarily recruited in the LUMC, and, if needed, from other hospitals by using advertisements. The study population will consist of 16 idiopathic stable PD patients with motor complications despite adequate medication. In- and exclusion criteria are reported in Table 2. During the study, PD patients are allowed to increase or decrease the dosage of medication or change the type of medication if needed. This will be taken into account for the analysis and interpretation of the study results.

All subjects will receive one FMT. To assess the variability of the study endpoints and to provide self-control data, two standard-of-care measurements (baseline and V3) will be performed before FMT.

The Netherlands Donor Faeces Bank (NDFB - http://www.ndfb.nl/), located in the LUMC, provides ready-to-use quality assured faecal suspensions from healthy donors for FMT in the Netherlands. General protocols for screening of donors and preparation of faecal suspensions in use at the NDFB have been described before.[29,51] Importantly, persons with constipation cannot become a donor and donors are asked whether there are any genetic diseases in the family. Two donors will be selected from the donor pool of the NDFB to minimise the risk of no or a negative response due to donor specific characteristics and to explore which donor gut microbiota characteristics are beneficial for PD patients. The donor selection will be randomised and double-blind.

An employee of the NDFB will use the cloud-based Castor clinical data management platform to produce a randomization list for the two donors using a variable block randomization method, that will not be disclosed to the investigators (that enroll participants)/physicians/patients involved in this trial. A technician will be informed of the outcome of randomisation for each patient, will prepare the selected material and will ensure that the syringes with the faecal suspension do not contain donor identifying information.

The randomization code could be broken in case of suspected FMT-related infections or adverse reactions, or when the Data safety monitoring Board (DSMB) deems it necessary.

Table 2. In- and exclusion criteria for patients with PD to participate in the FMT4PD study.

Inclusion criteria:	Exclusion criteria:
Clinical diagnosis of idiopathic PD according to UK brain bank criteria.[47]	Hoehn and Yahr scale stage 5 (most severe stage in scale for severity of PD motor symptoms).[48]
PD disease duration of at least five years.	Comorbidity or condition impairing ability to participate in the study according to the investigators.
Use of levodopa.	Current use of probiotics or in the previous three months.
Presence of motor complications (motor fluctuations or dyskinesias) despite adequate PD medication and regardless of severity.	Unstable PD with change in type or dose of PD medication in the previous three months.
Written informed consent.	Symptoms of a GI infection during the previous three months.
	Current need of antibiotics or use in the previous three months.
	Current GI malignancy or in the previous six months.
	Known obstructions, paralysis or severe motility problems of the gastrointestinal tract.
	Severe dysphagia with incapability of swallowing 2 liters of macrogol + electrolytes or inability to receive oral feeding.
	Known diagnosis of Inflammatory Bowel Disease[49] or celiac disease.[50]
	Intestinal resection in medical history.
	Recent intraabdominal surgery(< 3 months).
	Platelet count < $70x10^9$/L.
	Participation in another study within 16 weeks of screening visit.
	Known severe food allergy or allergy to medication that a donor could have used (intake may lead to a life threatening situation).
	Immunocompromised state. Current use of immunosuppressants or opiates, or in the previous month.
	For women with child-bearing potential: pregnancy; current wish to be pregnant or absence of contraception; lactation.

Table 2. *(Continued)*

Inclusion criteria:	Exclusion criteria:
	Impaired ability to understand the study content and to give written informed consent.
	Unwilling or not capable to comply with the study requirements.
	Inability to communicate in Dutch.

Abbreviations: GI: gastrointestinal, PD: Parkinson's disease, UK: United Kingdom.

Sample size

SAEs definitely or probably related to FMT have been reported in 0-5% of the patients with indications other than PD.[27-29] Since this is a pilot study, only 16 patients will be included. This is the number that is needed to have >80% chance that any FMT-related SAEs, that occur in >10% of the cases, might occur in the current study population. The occurrence of FMT-related SAEs in >10% of the PD patients is deemed useful information that might change the design of a future clinical trial or might result in the choice not to perform such a trial.

Study procedures

Screening

Selected patients will receive a patient information letter. If interested, the patient will be further informed on the study during the first visit. The research physician will determine whether the patient meets the in- and exclusion criteria and is able to participate. If the patient agrees to participate, the informed consent form will be signed in presence of the investigator. Thereafter, blood and clinical questionnaires concerning sociodemographic variables, present and past medical history and medication use will be collected. If participants give permission for the LUMC Biobank Parkinson, their blood samples (and some DNA from the blood) will be stored for indefinite duration for future (yet unknown) analyses. The final eligibility of the patient will be discussed in the "Parkinson working group", including at least one infectious disease specialist, gastroenterologist, medical microbiologists (FMT experts) and neurologist (PD expert).

Clinical evaluations

In the three days before the baseline visit, the patient will fill in a diary to describe the motor complications during the day. The day before the baseline examination, the patients will fill in a questionnaire, including questions on health status, diet, constipation, disease-related variables, medication use and motor and non-motor symptoms during the week previous to the visit. Furthermore, on the day of the visit, the investigators will complete an additional baseline questionnaire with more detailed questions about health

status, disease-related variables (motor and non-motor symptoms) and medication use and will perform a physical examination. Patients will be instructed to report all SAEs immediately to the investigators during the study period. (S)AEs will also be assessed at each visit using a standardised form. During the standard-of-care visit and post-FMT follow-up the same evaluations will be repeated (except for the baseline questionnaire). Additional blood samples will be collected at one week and three months post-FMT. During the last visit, the study load will be assessed.

Stool sampling

Stool samples are collected for analysis and evaluation of the FMT treatment effect and (S)AEs. The baseline stool sample, including all faeces from one defaecation collected in a faecotainer within four hours after defaecation before the baseline visit, will also be used for the preparation of an autologous faecal suspension to be stored for a potential rescue FMT. Additional stool (using faeces collection tubs) will be collected at one week after baseline (standard-of-care visit), and one week, six weeks, three months and twelve month post-FMT. Patients will be requested to collect stool samples of each defaecation from three days before a study visit, or earlier if the patient has severe constipation, until the visit, and to store it in the refrigerator. The most fresh stool sample will be delivered to the laboratory at the regular study visit. At six weeks post-FMT, patients will be requested to send a stool sample by mail, as soon as possible after defaecation, with storage in the refrigerator until transport. The stool samples will be stored and can be retrieved for microbiota analysis, culturing purposes, safety reasons (SAEs) or future research purposes.

Faecal suspensions and stool samples are stored in a -80°C freezer. The suspension for FMT contains 198 ml and is derived from 60 gram faeces with added glycerol up to a percentage of 10% as cryoprotectant. Autologous suspensions are allowed to be 99 ml, which means that if the baseline stool sample does not contain at least 33 gram, the patient will be asked to collect another stool sample. For the other stool samples, at least two times 1 gram faeces is stored with 10% glycerol for culturing purposes and at least two times 1 gram faeces is stored for microbiota analysis. In addition, when there is more faeces left and if participants give permission for LUMC Biobank Parkinson storage, two aliquots of 1 gram with 10% glycerol and two aliquots of 1 gram without glycerol will be stored in the LUMC Biobank Parkinson for future research purposes. Stool samples for this study will be destroyed 20 years after end of the study or for indefinite duration when stored in the LUMC Biobank Parkinson. The faeces consistency for every stool sample will be registered by the patient and the investigator using the Bristol stool scale.

FMT procedure

The patients will receive a healthy donor FMT in the hospital via direct injection into the horizontal duodenum through a gastroscope. Defrosted ready-to-use faecal suspensions

will be provided by the NDFB. The pre-treatment includes 2 liters of laxatives (macrogol + electrolytes) on the day prior to FMT, and vancomycin 250 milligram (mg) four times per day for five days until 24 hours before FMT.[51] In case of obstipation, additional laxatives (Bisacodyl, maximum 2 times 5 mg per day) will be administered in the two days before FMT to improve the efficacy of the bowel lavage. When this is not contraindicated, one pill of domperidone 10 mg will be administered orally on the day of FMT prior to FMT, to prevent nausea and to improve gastric motility. Domperidone could also be used after FMT, in case of nausea or vomiting. When preferred, mild sedation by intravenous administration of 0.5–7,5 mg midazolam before or during gastroscopy can be provided. The post-FMT observation period with regular vital parameter checks in the hospital will be at least two hours.

Outcomes

Study parameters/endpoints are shown in Table 3.

Table 3. Study parameters/endpoints

Main study parameters/endpoints
1. Feasibility of FMT in PD patients, assessed by the registration of the number of included patients that cannot undergo FMT due to a patient- or procedure-related reason at V4 (in case of >20% of patients that cannot undergo FMT, the FMT-procedure is considered not feasible).
2. Safety of FMT in PD patients, assessed by the registration of FMT-related SAEs at all post-allocation visits/telephone appointments (an FMT will be considered unsafe in PD patients, when there are definitely FMT-related SAEs in >10% of the cases).
Secondary study parameters/endpoints
1. Alterations in patients gut microbiota structure (16S rRNA gene amplicon sequencing) after FMT, with comparison to the donor gut microbiota, and how these associate with PD symptoms and motor complications (by collecting stool samples at V2,V3,V5, Tel2, V6 and V7).
2. Changes after FMT (as compared to the change observed after one-week standard-of-care observation) and differences between patient groups based on the selected donors on the following aspects (all visits and/or telephone appointments, except for V1 and V4): • Severity of motor complications, i.e. number and duration of "off" periods* and periods with troublesome dyskinesias per day (3 days diary) • MDS-UPDRS (on medication)[45] • Required PD medication dose • Hoehn and Yahr score[48] • Q10 questionnaire (wearing off)[44] • MOCA[46] • Severity of GI symptoms and defaecation frequency • Bristol stool scale • Other non-motor symptoms (SENS-PD)[43]
3. Ease of the study protocol, assessed by the reasons for refrainment of participation in the study after receiving full information at V1, and study load for participants, assessed by a 1-10 scale and open questions at V6.

Table 3. *(Continued)*

Secondary study parameters/endpoints
4. FMT-related AEs in PD patients after FMT, assessed by the registration of FMT-related AEs at all post-allocation visits/telephone appointments.
Other study parameters (at all visits and telephone appointments, except for V1 and V4)
• Sociodemographic factors • Diet • Health status • Disease characteristics

* 'Off' periods: a certain amount of time between regular doses of Parkinson medications, when the symptoms re-emerge or worsen. Abbreviations: FMT: faecal microbiota transplantation, MDS-UPDRS: Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale, MOCA: Montreal Cognitive Assessment, PD: Parkinson's disease, (S)AEs: (serious) adverse events, SENS-PD: SEverity of Non-dopaminergic Symptoms in Parkinson's Disease, Tel: telephone appointment, V: visit.

Data visualisation and analysis

For this study, both an intention-to-treat principle (ITT) and a per-protocol analysis will be conducted. Continuous variables will be summarised with means (with standard deviation) or medians (with interquartile range) and categorical variables with frequencies and percentages. If possible, ordinal outcomes on one subject will be summed. A two-tailed $p<0.05$ will be considered statistically significant. For linear mixed models, data will be converted into a logarithmic form in case of a skewed distribution. The investigators will attempt to prevent or minimise missing values by calling patients before every visit to remind them they need to fill in questionnaires and/or collect faeces and also every filled-in questionnaire will be checked on completeness. Linear mixed models and generalised estimating equation (GEE) take missing values into account, when data are missing at random. When applicable, Bonferroni corrections will be applied. After analysis of study results, unblinding of donor selection will be performed. This pilot study focusses on feasibility and safety as primary outcome and is not powered for the secondary outcomes.

The assessment of FMT feasibility and safety, the ease of the study protocol and FMT-related AEs will be descriptive.

The bacterial fraction of the gut microbiota will be profiled via 16S rRNA gene amplicon sequencing. DNA will be extracted from 0.1 gram faeces using the Quick-DNA™ Faecal/Soil Microbe Miniprep Kit (ZymoResearch, CA, USA). The V3-V4 or V4 region of the 16S rRNA gene will be sequenced on an Illumina platform. Raw sequencing data will be processed using a validated computational pipeline (NG-Tax,[52] Qiime2[53]) using the Silva 132 SSU database for taxonomic classification.[54] 16S rRNA gene amplicon sequencing sequence data of the gut microbiota of donors and patients of before and at several time points after FMT will be assessed for FMT-dependent changes in gut microbiota composition. Sequence reads will be clustered on similarity (100%[55]) and assigned to the

nearest bacterial phylum/family/genus and the relative abundance will be determined. Differences in bacterial diversity within and between samples will be evaluated by calculating the alpha- and beta-diversity of each sample. FMT-dependent changes will be defined as an alteration of alpha- or beta-diversity towards that of the donor and/or taxa abundances that become more similar to the donor microbiota after FMT. For gut microbiota analysis or continuous variables in the clinical data, outcomes post-FMT at several time points will be compared to pre-FMT data by linear mixed models (including one or (a mean of) all pre-FMT and one or all post-FMT measurements). Continuous variables may be converted into categorical variables. For categorical variables, generalised linear mixed models and/or GEE will be used (including one or all pre-FMT and one or all post-FMT measurements). A donor effect can be added, when applicable (or the Metagenomics Longitudinal Differential Abundance Method will be used).

The main outcome point is 1 week after FMT. All changes in clinical values and microbiota recorded at this time point with respect to baseline, will be compared with changes recorded at the standard-of-care visit (1 week after baseline).

Patient and public involvement

The first draft of the study protocol was reviewed by two "patient-researchers" of the Dutch Parkinson patients association and later discussed in person. Issue discussed included, safety issues and patient-centered value, the total study load, the burden of the intervention and time required to participate, choice of some procedures, and potential confounders of the outcome measures. Based on their advice, some changes were done to the original study design, such as two visits were replaced by telephone appointments to lower the burden for patients, and the number of ratings on the day of treatment was reduced to a minimum due to the possible stress related to the treatment. Furthermore, after the protocol was modified based on requirements of the Medical Ethical Committee, the final version was sent to the patient researchers for approval (Supplementary File S4). At the end of the study they will be involved in the interpretation of the results and consulted regarding the interpretation of potential adverse events and their relatedness to the procedure.

The Dutch Parkinson patients association has co-funded the study, which has been highlighted on their website. Results of the study will be propagated to the end users via their channels.

This study will see the participation of PD patients as study subjects.

Ethics and dissemination

Data collection and management

All PD patients will receive a pseudonymised study ID after signing the informed consent form. All clinical data and samples will be stored linked to this study ID. This study ID is linked to patient identifying data in a separate document, which will be securely stored on another password-protected location than the clinical research data. Completed patient questionnaires and diaries will be collected on paper and stored in a secured environment at the LUMC. These data and the results of the investigator examinations during visits or phone interviews and the blood analysis will be entered into a password protected cloud-based database at the LUMC (Castor) with real-time edit checks and automatic data saving. This database is only accessible for the study investigators, DSMB, monitors, and authorities for inspection of research. The raw 16S sequencing data of the stool samples will be stored in a folder with restricted access, and will anonymously be submitted to a public repository (European Nucleotide Archive). Data collection and storage and overall study procedures will be monitored by independent LUMC study monitors. Furthermore, independent GRP audits are regularly performed in the LUMC (https://www.lumc.nl/research/grp-and-integrity/grp/). The study results will be disseminated through publication in peer-reviewed journals and through presentations at international conferences. Authorship criteria are based on the International Committee of Medical Journal Editors (ICMJE).

Safety considerations

Prior to the start of the study, an independent DSMB will be assembled, consisting of two FMT experts (one gastroenterologist and one infectious disease specialist), one neurologist and a statistician. The DSMB will regularly meet to discuss all aspects of subject safety. The DSMB will perform an interim analysis when the first six patients have completed their six weeks post-FMT follow-up. The results will be disclosed to the investigators. In case of an SAE or on request of the investigator, the DSMB will be consulted to evaluate the relation with FMT and/or the potential need to terminate the study. The study will be terminated when there are definitely FMT-related SAEs in >1 patients at the interim analyses and/or when the subjects health or safety is jeopardised according to the DSMB, medical ethical committee and/or investigator. The principal investigator can also decide to withdraw a subject from the study for urgent medical reasons. Furthermore, patients are free to interrupt their participation in the study at any moment. The LUMC has a liability insurance and an insurance to cover health problems of participants caused by the study.

FMT is routinely performed in patients with multiple relapsing CDI, for whom it is considered a relatively safe procedure. A study performed by the NDFB on FMT-treated

patients with recurrent CDI revealed that approximately 21-33% of patients report mild gastrointestinal adverse events, such as abdominal pain and diarrhea, in the three weeks after FMT and at long-term follow-up.[29] Among patients that receive FMT for other indications than CDI, the percentage that develop these gastrointestinal adverse events is unknown. In 0-2% of patients receiving FMT via upper GI route, SAEs are reported which are probably or definitely related to the FMT or to the procedure.[28,29] Described SAEs that are possibly attributable to FMT or to the procedure via upper GI route include aspiration pneumonia, septicemia or other infections, fever, systemic inflammatory response syndrome, peritonitis, upper GI hemorrhage or death.[27-30,56,57] Long-term SAEs are largely unknown, although one recent study suggests FMT does not cause long-term SAEs.[58] No clinical trials have been performed with FMT in PD patients so far. In the available case series (total of 33 patients), one patient reported an SAE (episodes of vasovagal pre-syncope),[39] while mild transient side effects related to the procedure were reported in two series.[38,40] The incidence and type of FMT- or procedure-related problems and (S)AEs in this group is unknown, and will be the main objective of this pilot study.

(S)AEs after FMT will be monitored very closely by measurement of hemoglobin, platelets, inflammation parameters, liver enzymes, kidney function and electrolytes before and after FMT and by the use of standard questionnaires. Furthermore, the patient will be instructed to always contact the investigators immediately by phone or e-mail in case of any SAE (by phone outside working hours). Participation in the study will be recorded in the electronic medical record of the LUMC and patients will receive a information card with study information and contact details, enabling other physicians to contact the investigators. All (S)AEs will be followed until they have abated, or until a stable situation has been reached, also after withdrawal. In case of an SAE, the investigators will report this as soon as possible to the Parkinson working group and the DSMB. In case of definitely FMT-related SAEs, the Parkinson working group will decide whether it may be useful to perform an autologous rescue FMT and/or provide antibiotics, as this may potentially reverse the donor FMT effect.

In this pilot study, the upper GI route will be used for FMT. Aspiration of donor faecal material in patients without PD resulting in fatal aspiration pneumonia has been described in only a few cases.[27,28,59] PD patients with severe swallowing problems or decreased GI motility will be excluded from the study. In addition, faecal suspensions will be injected slowly and the patient will be positioned in upright position to prevent regurgitation. Domperidone will be used to prevent and/or treat nausea and to improve gastric motility.

Ethical considerations

The study will be conducted according to the principles of the Declaration of Helsinki[60] and in accordance with the Medical Research Involving Human Subjects Act (WMO).[61]

This study was approved by the Medical Ethical Committee Leiden Den Haag Delft (Supplementary File S4). Potential protocol amendments will be notified to this committee.

Status and timeline

The study has started in December 2021 and is expected to end in December 2023.

Discussion

Since there are no curative treatments available for PD and most PD patients with advanced disease experience less effectivity and/or adverse effects of PD medication, the development of new treatment strategies is highly desirable. Animal studies suggest a potential role of the gut microbiota in PD pathophysiology via the gut-brain axis and in the metabolism of levodopa, the mainstay of PD treatment. The most extreme form of modifying the gut microbiota is replacing the existing dysbiotic gut microbiota with a new normal microbiota from healthy donors. So far, no results of clinical trials on FMT in PD patients have been published and a pilot study to assess the safety and feasibility of FMT in PD patients appears a logical next step. The results may also provide some preliminary information on the efficacy of FMT in decreasing motor- and non-motor symptoms and motor complications, useful to design future studies. Analysis of the gut microbiota composition will reveal preliminary data on associated key taxa of the gut microbiota in PD patients with motor complications.

This pilot study has strengths and limitations. Strengths of this study are the use of two different donors, the broad range of clinical rating scales for Parkinson symptoms and constipation, the strict surveillance of (S)AEs, the inclusion of a standard-of-care measurement for comparison of the recorded changes, the analysis of the gut microbiota at different time points and the storage of an autologous suspension for treatment of a potential FMT-related SAE. Limitations include the absence of a comparator arm with placebo treatment, which was deemed too burdensome for the patients, considering the main focus on safety as outcome. In case FMT appears feasible and safe in this patient group, a larger double-blind randomised clinical trial may be performed to further explore the potential benefits of FMT.

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Author contributions

The study was conceived and supervised by MFC and EJK. KEWV coordinated the preparation of the study and wrote the study protocol. VOC supported KEWV in the literature search, the preparation of the study and will execute the study. EMT is head of the Netherlands Donor Feces Bank, provides the fecal suspensions for this study, provided advice on the protocol, coordinates stool sample collection and the FMT procedure. JJvH provided advice on the protocol. The FMT4PD study group (Supplementary File S2) provided advice and/or helped in the preparation of the study. All reviewers critically reviewed the manuscript.

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Competing interests

None declared.
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