**Clinical trial protocol**

Project title

**Faecal microbiota transplantation for patients with diabetes mellitus type 1 and severe gastrointestinal neuropathy: a randomised, double-blinded safety and pilot-efficacy study**

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# Summary

In this investigator-initiated randomised, double-blinded clinical trial, we aim to investigate the safety and pilot-efficacy of faecal microbiota transplantation (FMT) as an adjuvant to the treatment of severe gastrointestinal neuropathy in patients with diabetes mellitus type 1 (DM1). The study results will provide the information necessary to design a sufficiently powered clinical trial with FMT in this debilitating condition.

DM1 is often complicated by gastrointestinal symptoms such as diarrhea, nausea, vomiting, abdominal pain, constipation, and faecal incontinence. These may be caused by intestinal neuropathy which predisposes to abnormal amount and composition of microbiota in the gut. FMT from a healthy donor to a patient could potentially change the microbiota in the gut and reduce gastrointestinal symptoms in DM1. FMT aims to restore a disrupted gut microbiota and amend imbalances through establishment of a stable, complex microbiota.

The clinical trial will be conducted in accordance with this protocol, the principles for Good Clinical Practice (GCP), and the Declaration of Helsinki. It will be initiated following approval from the Central Denmark Region Ethical Committees and the Danish Patient Safety Authority. If the protocol is approved and permission to start patient inclusion is issued by the GCP Unit, patient inclusion may begin after 01 January 2021 and last until 31 December 2023.

# Abbreviations

| | |
|---|---|
| AE | Adverse event |
| AUH | Aarhus University Hospital |
| BS | Bristol stool chart |
| CDI | *Clostridioides difficile* infection |
| CEFTA | Center for Faecal Microbiota Transplantation |
| CTT | Colorectal transit time |
| DM1 | Diabetes mellitus type 1 |
| eCRF | Electronic case report form |
| EPJ | Electronic patient journal |
| FMT | Faecal microbiota transplantation |
| GI | Gastrointestinal |
| GCP | Good clinical practice |
| GET | Gastric emptying |
| GSRS-IBS | Gastrointestinal syndrome rating scale - irritable bowel syndrome version |
| IBS-IS | The irritable vowel syndrome impact scale |
| ICF | Informed consent form |
| PAGI-SYM | Patient assessment of upper gastrointestinal symptom severity index |
| SADE | Serious Adverse Device Effect |
| SAE | Serious adverse event |
| SBTT | Small bowel transit time |
| SOP | Standard operating procedure |
| TGA | Transglutaminase antibody |
| TMF | Trial master file |
| WMC | Wireless motility capsule |

# 1.0 Original title

Faecal microbiota transplantation for patients with diabetes mellitus type 1 and severe gastrointestinal neuropathy: a randomised, double-blinded safety and pilot-efficacy study.

# 2.0 Purpose of the study

## 2.1 Aim

The aim of the study is to evaluate the feasibility, safety and pilot-efficacy of faecal microbiota transplantation (FMT) as a treatment of severe gastrointestinal neuropathy in patients with diabetes mellitus type 1 (DM1).

## 2.2 Problem statement

DM1 may cause damage to nerve cells in the gut causing neuropathy that leads to changes in gastric and intestinal motility. This change predisposes to an abnormal amounts and composition of bacteria in the gut, probably leading to uncontrollable diarrhea and severely impaired quality of life. Transferal of intestinal microbiota from a healthy donor to a patient is called FMT. FMT may potentially change the bacteria in the gut and reduce gastrointestinal symptoms. However, FMT may also have potential side effects, especially in persons with autonomic neuropathy and delayed transit through the gut.

## 2.3 Hypotheses

### 2.3.1 Primary $H_0$

1. Oral intake of FMT capsules is safe and well tolerated in patients with DM1, including those with prolonged gastric emptying and small intestinal transit.

### 2.3.2 Secondary $H_0$

2. Oral intake of FMT changes the microbiota of the colon and the effect lasts for at least 4 weeks.
3. Oral intake of FMT is superior to placebo in reducing gastrointestinal symptoms in patients with DM1.
4. The effect of two doses of oral intake of FMT (separated by 4 weeks) is superior to one dose.

## 2.4 Endpoints

### 2.4.1 Primary endpoint

Number of adverse events of severity grade 2 or more assessed by CTCAE v5.0 during the first week after first intervention (FMT or placebo).

### 2.4.2 Secondary endpoints

Patient-reported outcomes are obtained from the bowel habit diary. Each patient fills out the diary every day for one week at baseline, for one week starting at each day of the two interventions

and for one week at the follow-up at week 26. Bivariate comparions are made, comparing differences between FMT and placebo at a) Baseline vs first intervention, b) Baseline vs second intervention, c) Baseline vs longterm follow-up (week 26) for the following variables:

1. Median stool consistency (Bristol scale)
2. Number of passages of hard stools (BS 1-2)
3. Number of passages of liquid stools (BS 6-7)
4. Median number of bowel openings per 24 hours
5. Number of nightly bowel openings (from bedtime until morning)
6. Number of episodes with involuntary defaecation
7. Glycemic control measured by patient reported use of insulin (IE)

Patient-reported measures from the schedule of side effects and telephone call 1 week after each intervention

8. Mild adverse events (grade 1) following FMT or placebo assessed by CTCAE v5.0
9. Adverse events in week 5-8
10. Serious adverse events during longterm follow-up

Patient-reported outcomes from questionnaires completed at baseline and 4 weeks after each intervention period and at 26 weeks follow-up. Bivariate comparisons baseline vs week 4, baseline vs week 8, baseline vs week 26:

11. Change in Gastrointestinal syndrome rating scale – irritable bowel version questionnaire (GSRS-IBS)
12. Change in patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM)
13. Change in irritable bowel syndrome impact scale (IBS-IS)

Objective measures from the wireless motility capsule performed at baseline and 4 weeks after each intervention period:

14. Transit time through the small intestine
15. Colonic transit time
16. pH drop from the small intestine to the colon

Objective measures from the low-dose CT scan performed at baseline and 4 weeks after the first intervention period:

17. Volume of the a) small intestine and b) the colon
18. Volume of gas in a) the small intestine and b) the colon

Objective measures from the breath test performed at baseline and 4 weeks after the first intervention period:

19. Rise in hydrogen PPM measured in breath test for small intestinal bacterial overgrowth

Microbiota analysis on faecal samples collected at baseline and 4 weeks after each intervention period:

20. Alpha-diversity of faecal microbiota, 16S
21. Dysbiosis index

Blood samples. Comparisons are made, comparing differences between FMT and placebo at a) Baseline vs first intervention, b) Baseline vs second intervention,
22. Glycemic control measured by HbA1C levels

## 2.5 Rationale

In patients with DM1 and severe gastrointestinal neuropathy, FMT could potentially change the composition of bacteria in the gut and reduce symptoms without the use of antibiotics. However, the use of FMT may have potentially serious side effects in patients with reduced gastric emptying, prolonged transit time through the small intestine, increased permeability of the gut and reduced resistance to infections. To date, no studies investigated the effects of FMT in patients with DM1 and severe gastrointestinal neuropathy**.** Therefore, it is necessary to evaluate the safety and pilot efficacy in a small group of patients before designing a large trial.

If this method is both acceptable, safe, and effective, it may provide a simple and antibiotic-free way of treating gastrointestinal symptoms in DM1 in an out-patient setting. This study will provide data on safety, tolerability and pilot efficacy which is an absolute necessity for further research within the field.

## 2.6 Background

### 2.6.1 Severe gastrointestinal neuropathy in diabetes mellitus

DM1 is strongly associated with diarrhea, nausea, vomiting, abdominal pain, bloating, constipation and faecal incontinence(1, 2). Though the severity of symptoms varies, severe cases suffer from daily uncontrollable diarrhea, vomiting, intense nausea, and faecal incontinence with severe consequences for social activities and quality of life(3). Additionally, nightly uncontrolled bowel movements pose a clinical challenge.

Earlier studies of patients with DM1 have demonstrated demyelination, axonal damage and reduced number of motor fibers in vagal nerves(4-6). Other studies report degeneration of nerve fibers within the enteric nervous system, including the Cajal cells (the pacemaker cell of the bowel)(7, 8). Today, it is assumed that diabetic gastrointestinal (GI) dysfunction is caused by autonomic neuropathy, dysfunction of the Cajal cells, and reduced contractility of the intestinal smooth muscle cells(7-9). The clinical effect of each component remains unknown.

### 2.6.2 Intestinal microbiota in diabetes mellitus type 1

Several studies have compared the gut microbiota of patients with early DM1 or even at a prediabetic stage with those of healthy volunteers. Findings have not been conclusive, but most have found reduced diversity of the intestinal bacterial community and increased proportion of *Bacteroide*s(10). Preclinical studies support the hypothesis that specific features of the microbiota give rise to impaired intestinal permeability(11), which further influence T cell autoimmunity and B-lymphocytes.

This may lead to beta-cell destruction and DM1. However, most research on gut microbiota and DM has been in DM type 2. Thus, the impact of the microbiota in humans with DM1 and GI symptoms remains obscure

### 2.6.3 Abnormal gut microbiota and gastrointestinal dysfunction in diabetes

The pH within the colon is lower than in the small intestine. This is mainly because of fermentation of bacteria in the right colon. Recently, we have shown that the pH drop from the small intestine to the colon is larger in patients with DM1 and neuropathy than in healthy individuals(12). This may be a result of increased fermentation caused by an abnormal microbiota. Our partners at the Technical University of Denmark have found that the diversity and composition, as well as the metabolic activity of the colonic microbiota is strongly associated colonic transit time(13).

In a recent study, we found that patients with DM1 and severe GI symptoms accumulate faeces and excessive amounts of gas in the caecum(14). Hence, we speculate that diabetic intestinal neuropathy causes dysmotility and stasis of stools in the right colon, resulting in abnormal microbiota composition and increased gas production. The excessive amounts of gas produced by the bacteria will distend the caecum until it responds with a forceful contraction causing explosive bowel moments resulting in diarrhea. Moreover, the increased gas production will contribute to the severe abdominal bloating and pain commonly reported by patients with DM1.

In clinical practice, most patients with DM1 and severe GI neuropathy have prolonged or normal transit time through the colon. Hence, standard treatment with constipating agents aggravates other bowel symptoms, especially symptoms caused by gastroparesis. The only treatment with a reasonable degree of success is repeated cycles of broad-spectrum antibiotics aimed to reduce bacterial overgrowth. Unfortunately, the effect is temporary and complications are common.

### 2.6.4 Faecal microbiota transplantation in patients with diabetes mellitus type 1

FMT is a method used to induce permanent changes of the gut microbiota. The microbiota from minimally processed faeces, containing whole microbial communities from a healthy donor, is transferred to a recipient and drives a prompt engraftment of a donor-like microbiota in the recipient(15). FMT is an established and efficient treatment to recurrent infections with *Clostridioides difficile* infection (CDI) with cure rates above 90 %(16-19). Most of our knowledge about the safety of FMT relies on studies with patients with recurrent CDI, and these studies often include patients with a high age and comorbidity. Even in these patients, FMT is well tolerated(20).

The Center for Faecal Microbiota Transplantation (CEFTA) at AUH is among the foremost internationally within the clinical use of FMT(21, 22). Recently, we developed FMT capsules for oral intake. This application method is simple, with low risk and is able to induce longterm changes in the microbiota(23). In patients with DM1, this may potentially reduce diarrhea, bloating and pain, without massive use of antibiotics. Simple and appealing as it may seem, the use of FMT may have unwanted consequences in patients with DM1. Reduced gastric emptying, prolonged transit through the small intestine, increased permeability of the gut and reduced resistance to infections could potentially predispose to serious side effects of FMT.

To the best of our knowledge, no studies of FMT in patients with DM1 and severe bowel dysfunction have been performed. It would therefore be unethical to introduce treatment or to conduct large trials without first ensuring the safety and tolerability in a small group of patients. Furthermore, the design of a regular and sufficiently powered trial requires pilot data on the efficacy of treatment and information on whether a single dose is sufficient to cause changes in the gut microbiota. Based on the above considerations, we believe that FMT holds great promise in DM1 enteropathy, but a thorough study providing data on safety, tolerability and pilot efficacy is an absolute necessity for further research within the field.

### 2.7 Previous study

Interest in FMT to treat disease has risen over the last few years and its therapeutic benefit is currently being explored for a variety of diseases. Numerous studies have explored the link between gut microbiota and DM1. However, there are no published studies investigating the safety of FMT against severe GI neuropathy in patients with DM1. Use of MeSH-band search is limited because FMT was first defined as a MeSH term in 2016. A PubMed search [2th October 2020] showed no previous study on the subject using the following search string:
[(((diabetes) OR diabetes type 1)) AND (((((faecal microbiota transplantation) OR fecal microbiota transplantation) OR faeces transplantation) OR human intestinal microbiota transfer) OR faecal bacteriotherapy)].

## 3.0 Methods and their justification

### 3.1 Study design

The study is a 8-week, randomised, double-blinded, placebo-controlled pilot trial of oral FMT versus placebo in patients with DM1 and severe GI neuropathy. The intervention period consists of a first 4 weeks where patients receive either FMT or placebo and a second 4 weeks where all patients receive FMT (Figure 1 and 2). The patients will undergo the investigations before and after each 4-week period. When the patients have completed the study period, they will be contacted by telephone every second month for two years. They will be asked about their gastrointestinal symptoms. The patients will be offered further treatment with FMT, if they experience relapse of symptoms and a score of or above 40 in the GSRS-IBS questionnaire.

The design is chosen to allow the evaluation of the safety and tolerability of FMT in patients with DM1. We will also be able to evaluate if FMT is superior to placebo, how it affects the intestinal microbiota and if the effects of two doses of oral FMT is superior to one dose.

### 3.2 Intervention

#### 3.2.1 Faecal microbiota transplantation

FMT may be administered via the upper route, using capsules or a nasojejunal tube, or via the lower route using colonoscopy. Oral administration with covered capsules is a patient-friendly and safe administration form, and it requires no specific patient preparation. At CEFTA, donor faeces is obtained from thoroughly screened healthy blood donors and processed in compliance

with the European Tissue and Cells Directive(21, 22). The faeces is minimally processed through a series of centrifugation steps and dispensed into double-coated, acid resistant enterocapsules (VcapsTM size 0 and 00, Capsugel®). A single treatment includes approximately 22 capsules (~50 grams of original donor faeces) each sized 0.5 x 2 cm. The capsule coating has a smooth surface making them easy to swallow. We have successfully applied FMT capsules in more than 200 patients with recurrent *Clostridioides difficile* infection, the main indication of FMT, with success rates comparable to other administration forms by naso-jejunal tube or colonoscopy(16) If a patient has completed the first two treatments with FMT capsules, and meets the criteria for further treatment, and the patient has had difficulty swallowing the capsules or have vomited during or after treatment with FMT, the patient may be offered treatment by colonoscopy or na-sojejunal tube.

#### *3.2.1.1 Clinical application of FMT*

Clinical treatment takes place in the Department of Hepatology and Gastroenterology and will be administrated according to National clinical guidelines(24) and the described standard protocol (FMT ud-af-huset-vejledning, appendix 1). Prior to clinical treatment, the FMT is released and dispensed from the freezer of the CEFTA laboratory according to a standardised release protocol compliant with safety standards for handling blood components. Safety samples are taken from all FMT treatments, and the samples are saved for later traceability and testing.

All patients arrive fasting (6 hours) for their randomised dedicated treatment. Treatment with capsule-FMT or capsule-placebo takes place in outpatient observation rooms. Prior to treatment, patients receive a single dose of peristaltic 10 mg tablet metoclopramide. The physician responsible for the treatment breaks the seal and the capsules with FMT or placebo are indigested with diet soda over 10-30 minutes. Outpatients then leave the ward to their own home.

## 3.3 Analyses

### 3.3.1 Assessment of gastrointestinal symptoms

a) Patients will fill in a daily bowel diary, commonly used at our unit and a side effect schedule which will be handed out to the patients in paper form. The patients will fill out the diary and side effect schedule for one-week diary four times: at baseline, during the first week of each intervention period, and at longterm follow-up after 26 weeks. The diary contains questions that include number of daily bowel movements, consistency of stools (Bristol stool chart), time spent at defecation, nightly bowel movements, episodes of faecal incontinence, days with abdominal bloating/pain and the amount of insulin used. The side effect schedule will contain a schedule of commonly known side effects and an assessment of the severity of the side effect (appendix 2).

The following four questionnaires will be filled out by the patient on an Ipad during the scheduled visits and the data will be saved directly in REDCcap.

b) GSRS-IBS: The Gastrointestinal syndrome rating scale - irritable bowel syndrome version (GSRS-IBS) is a 13-item questionnaire developed and validated for irritable bowel syndrome(25). The items cover some of the most common bowel symptoms experienced by patients with DM1. These include pain, bloating, diarrhea, constipation and gas. The questionnaire adds to a total score which covers the previous week. The questionnaire has been developed and validated in a Nordic country (Sweden), translated into Danish and subsequently used in several studies from our unit at AUH (appendix 3).

c) PAGI-SYM: The patient assessment of upper gastrointestinal symptom severity index (PAGI-SYM) questionnaire has been developed and validated for functional dyspepsia(26). The questionnaire is composed of 20 items with 6 subscales: heartburn/regurgitation, nausea/voliting, fullness/satiety, bloating, upper abdominal pain and lower abdominal pain. PAGI-SYM scores have been shown to correlate with gastric emptying rate and gastric hypersensitivity and therefore may be useful in the evaluation of gastroprokinetics(26) (appendix 4).

d) IBS-IS: The irritable bowel syndrome impact scale (IBS-IS) is a 26-item questionnaire developed to capture the impact of irritable bowel syndrome and its treatment on patients' lives(27). The 26-items represent five domains: Fatigue. Impact on daily activities, sleep disturbance, emotional distress and eating habits (appendix 5).

e) COMPASS-31 is a 31-item questionnaire for assessing symptoms of dysautonomia. The questionnaire has been validated in patients with small fiber polyneuropathy(28). It quantifies 6 domains: orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder and pupillomotor (appendix 6).

### 3.3.2 Wireless Motility Capsule

The Wireless Motility Capsule (WMC) consists of an indigestible capsule that continuously measures pressure, temperature and pH as it traverses the GI tract(29). Based on stereotypical landmarks, gastric emptying time, small bowel transit, colonic transit time and whole gut transit time can be delineated in a single recording (Figure 3). Data is transmitted wirelessly to a receiver unit worn by the patient until the capsule is expelled. Investigations on normal values have been performed and the WMC is CE marked and its use has been approved by the US Food and Drug Administration as well as the European Authorities. In a study among patients with DM1 experiencing GI symptoms, 65% had prolonged gastric emptying, 24% had prolonged small intestinal transit and 58% had prolonged colon transit time(29).

In the present study, assessment with the WMC will serve to classify patients into those with normal or prolonged gastric emptying/small intestinal transit at baseline. Further, the method will quantify changes in the pH of the colon (a marker of fermentation) from baseline to after FMT. Finally, the WMC will provide a direct measure of colonic transit time at baseline and after intervention with FMT. The WMC is available at our centers.

#### *3.3.2.1 System malfunction*

There has only been reported a small number of cases of system malfunction. These included inabilities of the patient to swallow the capsule, failure of the capsule to record or to transmit data, failure of the receiver to record or download data and software malfunctioning.

In a clinical trial with 495 subjects receiving WMC involving healthy, gastroparetic and constipated patients, 3 patients were unable to swallow the capsule, yielding a failure-to-swallow incidence rate of 0,6%. In the same clinical trial there were 36 (7,2%) incidences of equipment or software malfunctioning preventing the acquisition of interpretable transit data from the WMC(30). The trial involved prototypic equipment that has since been upgraded. In a postmarketing analysis of approximately 5,000 WMC ingestions, the incidence of equipment failure was reported to be 0.8-0.9%(31).

### 3.3.3 Low-dose abdominal CT scan

A low-dose high-resolution CT scan will be performed after a minimum of 6 hours fasting for food and a minimum of 2 hours for liquids. The scan field covers an area from at the left cardiac ventricle to the lower part of the anal canal allowing assessment of abdominal organ volumes as well as gas and fluid volumes within the gut. Data analysis will be performed in PMOD version 3.6 (PMOD Technologies, Zurich, Switzerland). Regions of interest are manually defined on each slice of the CT. Volumes-of-interest are computed by fusing all the regions of interest. Water/fluid is defined by Hounsfield CT unit < 30 for water and < -200 Hounsfield units for gas. The investigator making all analyses will be blinded to the clinical category of the test subject and whether the scan is performed at baseline or after intervention with FMT.

### 3.3.4 Breath tests

Due to the acidity of the stomach and the distally propagating small intestinal motility, the concentration of bacteria in the small intestine is low. This is in strong contrast to the colon where the concentration is extremely high. Increased numbers of bacteria in the small intestine may cause bloating, malabsorption and diarrhea. A simple diagnostic test for small intestinal bacterial overgrowth is the breath test whereby a substrate (80 g glucose dissolved in 300 ml of water) is swallowed. When the glucose gets into contact with bacteria, it is fermented and gas is produced, absorbed and excreted in the breath. The exhaled air is collected in small bags and analysed. An early peak of hydrogen or methane indicates small bacterial overgrowth. The method is simple and non-invasive, but results should be taken with caution because of several sources of error(32).

### 3.2.6 Stool samples

Stool samples are collected by the patient though the study period. Stool samples are used for both routine diagnostics and research purposes with later microbiome analysis.

### 3.3.6 Blood samples

Blood samples will be taken during the study period. There are indications that the gut microbiota may influence the glycemic control. The exploration of this is beyond the scope of the present study. However, blood glucose and HbA1c levels will be determined during the study.

### 3.3.7 Control of blood glucose before and during the investigations

The patients will be fasting for the investigation with WMC, low-dose CT scan, breath test and for the intervention with either FMT or placebo. The patients will be fasting from midnight the night before the day of investigation. Fasting takes place at home as the diabetic patients are used to monitor their blood glucose. The patients take their morning insulin and refrain from taking other oral medications. Blood glucose is measured once an hour while fasting in the hospital. If the blood glucose during the fasting period or during the study exceeds 10 mmol / l, the following regime with fast-acting insulin will be followed:

Blood glucose 11-12 mmol/l give 4 IU
Blood glucose 12-16 mmol/l give 6 IU
Blood glucose > 16 mmol/l give 8 IU

The interval between supplemental subcutaneous insulin should be 2-4 hours. Alternatively, the insulin can be administered intravenously. If the blood glucose falls below 5 mmol / l, 500 ml of 10% glucose is given on a side drop 25 ml/h.

## 3.4 Placebo

Placebo capsules will be produced in collaboration with the laboratory at the department of Hepatology and Gastroenterology, AUH. The placebo capsules are produced from a suspension of 50% glycerol, 40% sterile saline and 10% food coloring in enterocapusles to make them indistinguishable from the FMT capsules. All capsules are kept at -80°C until use. Processing and handling follow standards of the tissue and cell legislation. The capsules have no taste, are odorless, and are stored in vials labelled “study capsules.” The frozen FMT capsules and placebo capsules are identical in terms of visual appearance, weight, and vials and the final number of placebo capsules is varied between 10 and 30 to ensure blinding.

## 3.5 Randomisation

Randomisation will determine which group of patients will receive active treatment with FMT and which group will receive placebo capsules. Both participants and investigators will be blinded to the intervention.

Randomisation is performed by the project nurse at the Clinical Trial Unit at the department of Hepatology and Gastroenterology, AUH. The delivery is handled by the CEFTA laboratory staff after contact with the clinical staff. The randomisation list is established by the Clinical Trial Unit, Hepatology and Gastroenterology, AUH. Envelopes with procession information are packed according to the randomisation list. The CEFTA laboratory is continuously packing treatment boxes for each patient at randomisation according to the treatment with FMT + FMT or Placebo + FMT. The packaging of the boxes is standardised with double control and documented as a standard FMT for traceability as in routine FMT. The labeling on the packages does not re-

veal whether it contains placebo or FMT. Treatment boxes are marked with a number corresponding to the number drawn from the randomisation list, and at the start of the treatment it is ensured that these matches. The randomisation list is kept secret in the projects Trial Master File.

Premature unblinding is possible if investigators deem it necessary or in the event of conditions leading to interruption of the entire trial. This could be following several adverse events (AE) suspected to be in relation to active treatment with FMT, or if the trial terminates prematurely because of inability to include 20 patients. Any unblinding will follow the SOP "Kodebrud" and will be documented in the TMF.

## 3.6 Practical implementation and investigations

Twenty patients suffering from DM1 and severe GI neuropathy will be included in the study. The included patients will be identified during attendance in a specialised outpatient clinic in AUH to which they are referred to because of their GI symptoms. Prior to inclusion, standard clinical work-up including examination and blood samples have been performed to exclude competing diseases affecting the gastrointestinal system (celiac disease, thyroid disease, inflammatory bowel disease, infection and malabsorption). Colonoscopy will have been performed on clinical indication.

The trial will be performed at the Department of Hepatology and Gastroenterology at AUH. The participants remain outpatients and visit the department ten times. Investigations will be made at baseline and at the end of the two intervention periods.

**Table 1.** Schedule of procedures

| Week | -3 | -2 (+1) | -2 (+1) | 1 | 5 (+1) | 5 (+1) | 6 (+2) | 10 (+2) |
|---|---|---|---|---|---|---|---|---|
| | Information and inclusion | Baseline investigations (I) | Baseline investigations (II) | 1. Intervention | Investigations after 1. intervention (I) | Investigations after 1. intervention (II) | 2. Intervention | Investigations after 2. intervention (I) |
| Visit No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Inclusion | | | | | | | | |
| Information | x | | | | | | | |
| Informed consent | x | | | | | | | |
| In- and exclusion criteria | x | | | | | | | |
| Medical anamnesis | x | | | | | | | |
| Randomisation | x | | | | | | | |
| Exchanges | | | | | | | | |
| Equipment handed out | | x | | | | | | |
| Intervention | | | | | | | | |
| FMT or placebo | | | | x | | | | |
| FMT | | | | | | | x | |
| Questionnaires | | | | | | | | |
| Bowel diary | x | | | x | | | x | |
| GSRS-IBS | | x | | | x | | | x |
| PAGI-SYM | | x | | | x | | | x |
| IBS-IS | | x | | | x | | | x |
| COMPASS-31 | | x | | | | | | |
| Measurements | | | | | | | | |
| Wireless Motility capsule | | x | | | x | | | x |
| Low-dose CT | | | x | | | x | | |
| Breath test | | | x | | | x | | |
| Stool samples | | x | | | x | | | x |
| Blood samples | | x | | | x | | | x |
| Additional | | | | | | | | |
| Adverse events | x | | | | | | | |

### 3.6.1 Information and inclusion (visit 1)

Potentially eligible patients are informed about the project and offered ≥24h to consider participation. If the patients are willing to participate in the study, and they meet the in- and exclusion criteria, they will sign the ICF. If eligible, they will be assigned a unique study number and randomised to either active FMT or placebo. The patients will receive a bowel habit diary, a collection kit for faecal samples and a schedule for the study period.

### 3.6.2 Baseline investigations I (visit 2)

The patients arrive at the department of Hepatology and Gastroenterology AUH following an overnight fast (at least six hours). If relevant, a pregnancy test will be performed. The patients will be served a standardised meal before initiating the investigation with the Wireless Motility Capsule. Furthermore, the patients will fill out the questionnaires and blood samples will be drawn. The patients will be advised to fill out the bowel habit diary for the following 7 days.

#### *3.6.2.1 Timeline visit 2*

| | |
|---|---|
| 08.00-09.00 | Questionnaires |
| 09.00-09.30 | Standardised meal and WMC ingestion |
| 09.30-09.40 | Blood samples |

### 3.6.3 Baseline investigations II (visit 3)

The patients will arrive in the morning at the Department of Radiology at AUH for the second day of baseline investigations. They will arrive fasting (for at least six hours). The patients will undergo a low-dose high-resolution CT scan. The investigation takes approximately 30 minutes.

Afterwards the patients will go to the department of Hepatology and Gastroenterology to the investigation room at the second floor. The patients will undergo a breath test. Glucose is given orally and the patients will breathe into a breathalyser and the amount of methane and hydrogen in the exhaled air will be measured. The investigation takes approximately two hours.

#### *3.6.3.1 Timeline visit 3*

| | |
|---|---|
| 08.00-08.30 | Low-dose CT scan |
| 09.00-11.00 | Breath test |

### 3.6.4 1. Intervention. FMT or Placebo (visit 4)

The patients will receive treatment with either active FMT or placebo. The capsules will be administered by a nurse not otherwise involved in the study. The patients will continue their usual daily living while keeping a bowel diary for 7 days. They will furthermore be advised to fill out a daily report on side effects for 7 days. After 1 week, a telephone call will be made to ensure that no major side effects have occurred.

### 3.6.5 Investigations after 1. Intervention I and II (visit 5 and 6)

After 4 weeks since the first intervention, the investigations made at baseline will be repeated.

### 3.6.6 2. Intervention. FMT (visit 7)

During the second intervention period all patients will receive the active treatment with FMT. The following 4 weeks the patients will continue normal daily activities. They will be advised to fill out the diary and the report on side effect for 7 days following the FMT. A telephone call will be made after 1 week to follow-up on potential side-effects.

### 3.6.7 Investigations after 2. Intervention I (visit 8)

After an additional 4 weeks since the second intervention, investigations will be repeated once again except for the breath test and low-dose CT scan.

### 3.6.8 Follow-up post study

The patients will be offered further treatment with FMT (open label), if they have reported effect of the treatment, and they experience relapse of gastrointestinal symptoms. After completing the study period, the patients will be contacted every two months by telephone to follow up on their gastrointestinal symptoms. If the patients report relapse of symptoms, they will be asked to complete the GSRS-IBS questionnaire. If they achieve 40 point or above in the questionnaire, the patients will be offered treatment with FMT. The patients will also be asked to collect stool samples for microbiome analysis. FMT treatment may be repeated at later recurrence of symptoms, in the following two years, after the patients have completed the study. The follow-up post study is an additional option for the patients. They may at any time refuse to be contacted or to receive further treatment.

# 4.0 Statistical considerations

## 4.1 Sample size

This is the first study with FMT to patients with DM1 and therefore the assumptions for formal power calculations are not present. We expect that a group of n = 20 patients with DM1 is sufficient to determine whether there are any serious side effects associated to administration of FMT.

## 4.2 Statistical analyses

Descriptive statistics such as safety profile/adverse events and demographic data will be reported as the interquartile range (median ±IQR). Data from the WMC, Low-dose CT and breath test will be analysed using repeated measures regression with the intervention (FMT or placebo) as random effect.

The final statistical analysis plan, providing details of the analysis and presentation of results, will be finalised before initiation of any statistical analysis. Patients considered for the evaluation of safety and efficacy will be defined in the statistical analysis plan. Statistical analysis will be performed only after unblinding.

# 5.0 Participants

The Danish organisation “Diabetesforeningen” has estimated that there are 28.000 people in Denmark with type DM1. Several studies provide evidence that DM1 is associated with an increased prevalence of lower and upper gastrointestinal symptoms. However, the prevalence of patients with DM1 and severe gastrointestinal neuropathy is unknown. Judged from clinical experience in the tertiary centers at AUH And AAUH we believe it possible to find 20 patients who are willing to participate in the study.

## 5.1 Inclusion criteria

- Adult (≥ 18 years old), male or female patients with DM1 for at least 5 years

and

- Average of or above 40 points in the questionnaire: Gastrointestinal syndrome rating scale – irritable bowel syndrome version (GSRS-IBS).

## 5.2 Exclusion criteria

- Inability to understand Danish or the trial procedures
- Known or anticipated pregnancy (excluded by male sex, postmenopausal women and otherwise negative U-HCG)
- Known severe renal insufficiency (eGFR < 20 mL/min)
- Antibiotic use in the prior 4 weeks
- Patients with recent changes in morphine treatment (within the last 4 weeks)
- Ongoing infection with *Clostridioides difficile* or pathogenic intestinal bacteria or parasites (negative PCR test)
- Known gastrointestinal disease or GI infection (diagnosed with celiac disease, inflammatory bowel disease, infection and gastrointestinal cancer)
- Patients with dysregulated thyroid disease (abnormal TSH)
- Patients diagnosed with intestinal stricture
- Patients with other known disorder that can cause gastroparesis (ex. Parkinson’s and scleroderma)
- Patients with planned MR scan within 4 weeks
- Patients with pacemaker/ICD
- Previous abdominal surgery (minor surgical procedures ex. appendectomy allowed)
- Changes in medicine that affects the GI tract in the prior 4 weeks

## 5.3 Assessment of inclusion criteria

All patients will, prior to inclusion, have went through standard clinical work-up and have been seen in the outpatient clinic by a gastroenterologist. They have been examined and have taken blood samples to exclude competing diseases affecting the GI system. Colonoscopy will have been performed on clinical indication.

### 5.4 Trial termination, discontinuation, and replacements of patients

The investigation is terminated when 20 patients have completed the two intervention periods, or if investigators become aware of any conditions or events that suggest a possible hazard to the patients. Premature termination may also be a result of failure to recruit a sufficient number of patients. The trial is terminated when the data are analysed.

Participants will be discontinued if she/he withdraws her/his consent, in case of pregnancy, or intestinal surgery. Dropouts will be attempted replaced, until at least 20 participants have completed all three intervention periods. This can ensure a balanced number of patients receiving treatment. No more than 40 patients will be included in the trial, in the attempt to find 20 patients who, complete both intervention periods.

## 6.0 Risks, adverse events, and nuisance

We expect no long-term risks following participation. Side effects of FMT tend to be mild and short-lasting. Treatment with FMT is generally well tolerated, but specific side effects are sparsely elucidated. Experience has shown that about one-third of patients receiving FMT experience transient diarrhea, nausea or bloating(16, 33, 34). Less than 1 in 10 experience fever and discomfort in the hours after FMT. For most patients, the condition lasts less than a day and does not require treatment. The patient is carefully instructed after each treatment and receives information of how to contact the department in case of worsening or side effects. Approximately 1 in 20 develops symptoms compatible with bacterial overgrowth. These are treated according to local instructions of the department. Less than 1% of the patients receiving FMT experience severe long-term symptoms of infection with infectious disease from the donor. The risk is reduced as much as possible by thorough selection and screening of donors and donor faeces on accordance with tissue standards and the European Union Tissue and Cells Directives which leads to the safety standards comparable to handling of blood components. The application form of capsule FMT is minimally invasive. The inconvenience is ingestion of the many capsules and overnight fasting prior to ingestion of the capsules. Treatment with peristalsis-promoting medication helps in addition to accelerated emptying of the stomach for possible nausea.

Blood sampling may be associated with minor discomfort, bleeding, hematoma, and/or bruising, and there is a minimal risk of infection or inflammation, however this is easily treated. There are no known risks involved with faecal sampling.

The wireless Motility Capsule is a noninvasive and painless method of obtaining important data of the GI tract. We anticipate no adverse events (AE) caused by the Wireless Motility Capsule, however, there is a low risk of retention of the capsule. If a patient does not pass the capsule within 4 weeks, they will be offered an abdominal x-ray and possibly gastroscopic, colonoscopic or surgical removal of the capsule.

## 6.1 Safety precautions and monitoring

Only trained personnel will handle the equipment and perform the analyses. The project will be carried out according to the applicable required legislations. The GCP-unit at Aarhus University Hospital will monitor the project. Monitoring includes 100% of signed consent forms and serious adverse events (SAE).

### 6.1.1 Adverse events

Adverse events are defined according to the ICH Harmonized Tripartite Guide to Good Clinical Practice (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-e-6-r2-guideline-good-clinical-practice-step-5_en.pdf).

- Adverse Event (AE): any untoward medical occurrence in a patient which does not necessarily have a causal relationship with the trial
- Adverse reaction (AR): any adverse and undesirable reaction to the experimental treatment, regardless of dose.
- Unexpected adverse reaction (UAR): a side effect whose nature or severity does not match the expected side effects of the trial.
- Serious Adverse Event (SAE) or serious adverse reaction (SAR): any adverse event or adverse reaction that results in:
    1. death or
    2. a serious deterioration in health that
        - resulted in life-threatening illness or injury
        - resulted in permanent impairment of body structure or body function
        - required hospitalisation or prolongation of existing hospitalisation
        - resulted in medical or surgical treatment to prevent the above
        - foetal death, congenital anomaly or birth defect or any other negative effect on the foetus.
- Suspected Unexpected Serious Adverse Reaction (SUSAR): a suspected but unexpected serious adverse reaction that has not been previously described for the treatment with FMT according to published science.

All patients with expected or unexpected events or side effects are offered admission to the treatment ward if necessary. Furthermore, all unexpected side effects and AE will be reported in the "Adverse Event CRF" containing following information:

1. Degree of difficulty (mild, medium, severe)
2. Relation to the investigation (expected/unexpected)
3. Duration (start date, end date, are the condition persistent after the last examination)
4. The graduation of the adverse event according to the CTCAE v0.5

#### *6.1.1.1 Reporting adverse events*

Sponsor-investigator is responsible for the monitoring of possible unexpected side effects and AE occurring to patients in the investigation. All unexpected severe side effects (SUSARs) will

be reported to the Danish Patient Safety Authority who is the overall regulating authority for the research project, and to Ethics Committee. The Danish Patient Safety Authority will be notified by the electronic report form "reporting suspicion of serious adverse events during removal, testing, etc. of tissues/cells" (https://stps.dk/da/tilsyn/blod,-vaevceller-og-organer/vaev-og-celler/bivirkninger-og-uoenskede-haendelser/mistanke-haendelse/). If the SAE is categorized as a "Utilsigtet hændelse", it is reported to the Danish patient safety database (https://stps.dk/da/laering/utilsigtede-haendelser/), and a core cause analysis is carried out according to local standards at AUH. Once the core cause analysis is completed, the Danish Patient Safety Authority will receive the electronic report form "report conclusion of investigation of serious adverse events during removal, testing, etc. of tissues/cells" (https://stps.dk/da/tilsyn/blod,-vaevceller-og-organer/vaev-og-celler/bivirkninger-og-uoenskede-haendelser/konklusion-haendelse/).

All AE, AR, SAE and SUSAR are documented in patient-specific case report form (CRF) at the clinical attendances or by the telephone call that is made to each patient 1 week after each intervention. SUSARs occurring during the trial are reported as soon as possible and within 24 hours of notification. The project manager and sponsor-investigator are informed of all UAR, SAE and SUSAR and regularly review trial patients' CRF and are responsible for reporting.

Annually, safety reports are generated with an overview of all expected an unexpected serious incidents and side effects during the period, which are reported to the Danish Patient Safety Authority and the Ethics Committee. The annual safety report will contain information about potential AE in the trial period, evaluation of the severity and risks-benefit according to the AE. At the end of the project, an overall final report is made.

The connection to the study ends when a participant has completed the second intervention period, after approximately 12 weeks, or withdraws her/his consent. The patient may be followed up after the trial if an AE/SAE related to the study is not resolved when the intervention ends. In that case, the patient will be offered to be followed up by telephone until a solution is found or until it terminates.

#### *6.1.1.2 Interruption of the study*

If a SUSAR occurs, it is reported by the sponsor to the Danish Patient Safety Authority within 24 hours. The sponsor also assesses whether the trial should be interrupted or special precautions should be taken, for example in the form of exclusion of certain donors or change in the protocol. All changes are documented in the Trial Master File.

#### *6.1.1.3 Wireless Motility Capsule*

The WMC is an orally ingested data recording device that enables simultaneous assessment of regional and whole gut transit. The WMC is CE marked and approved by the US Food and Drug Administration for evaluation of patients with suspected delayed gastric emptying and evaluation of colonic transit time in patients with chronic constipation. The WMC is contraindicated to patients with a history of gastric bezoar, swallowing disorders, dysphagia to food or pills, suspected

stricture or fistulae along the GI tract, physiological GI obstruction, GI surgery within the previous 3 months, Crohn's disease, diverticulitis or an implanted or portal electromechanical medical device(31).

The most serious AEs associated with the WMC are the inability to confirm passage of the capsule outside the body, capsule retention and obstruction. In a previous trial with nearly 6000 patients receiving WMC there were 20 reports of prolonged capsule retention (5 in the stomach, 2 in the small intestine and 13 in the colon) which yield a retention rate of 0,33%(30). An upper endoscopy was required for extraction of the 5 capsules that were retained in the stomach. Of the remaining 15 cases, only 1 capsule required drug intervention. The other 14 capsules passed spontaneously. None of the patients required surgical intervention. If it cannot be confirmed that the capsule has passed five days after ingestion the manufacturer's follow-up recommendations should be followed. This is based on the location of the capsule which is determined by the pH profile obtained during the study. If the pH profile suggests that the capsule is retained in the stomach or small bowel, serial radiograph imaging at 3-week intervals is recommended until the capsule is retained in the stomach or bowel. Due to the low risk of obstruction once the capsule is in the colon, no specific follow-up is necessary if the capsule is retained in this area.

Other side effects associated with WMC include abdominal pain, dysphagia, nausea and diarrhea. However, as many of the patients had these symptoms at baseline, these side effects have been difficult to determine if they were caused by the WMC. Patients are provided information about the risks in the participants information sheet, which they are urged to read comprehensively prior to consenting to participate.

## 6.2 Radioactive exposure

A low dose CT scan involves radiation exposure. The measurement takes approximately 20 minutes and is planned to be performed twice per patient. In a low dose CT scan, the amount of radiation is around 0.83-2.0 mSv. This is classified as a "category IIb" radioactive exposure risk, which comprises a damage risk of 1 to 10,000. The exposure from two low dose CT-scans corresponds to the natural radiation you get in a little less than one and a half year from the surrounding environment in Denmark. The increased risk of developing cancer from two low dose CT Scans is 0.02%. The general risk of dying of cancer in Denmark is 25% and two low dose CT scans will increase this risk to 25.02%. The participants will be informed about the radiation exposure in the participant information sheet, and pregnancy is a contraindication.

# 7.0 Data collection

## 7.1 Biological material

In the project, biological material is taken in the form of faecal samples and venous blood. In total a maximum of 3 x 10 g = 30 g of faeces per included patient is stored. From each patient 3 x 4 ml = 12 ml venous blood is taken for the research project.

### 7.1.1 Blood samples

Blood samples will be collected at visit 3, 5 and 8 and analysed for:

- Blood glucose
- HbA1c
- Creatinine
- EGFR
- C-peptide

### 7.1.2 Faecal samples

The patients will be handed a collection kit at the inclusion and they will be advised how to collect the faecal samples and store them at home at -20 °C. They will collect the faecal samples in the days leading up to visit 3, 5 and 8 and bring them for their appointment. A validated collection kit (EasySampler®, GP Medical Devices ApS) is used and approximately 10 grams of faeces stored per attendance. Once the faeces samples have been delivered to the department of Hepatology and Gastroenterology they will be stored at -80 °C for microbiota analysis(35) which will be performed at the Technical University of Denmark, Lyngby.

## 7.2 Research biobank

All biological material collected during the investigation will be analysed within 5 days, and susequently destroyed, except:

- Stool samples will be frozen and stored for analysis of metabolites

These samples will be pseudonymised and stored in a research biobank at the Department of Hepatology and Gastroenterology at AUH until analysed. The European General Data Protection Regulation will apply and after analysis of metabolites have been performed, all biological material collected as part of the project will be destroyed. Handling and shipment of samples will be performed according to the Danish Data Protection Act.

## 7.3 Quality assessment

### 7.3.1 Source data

Source data will be registered in paper form or in EPJ. Data will then be stored in the electronic Case Report Form (eCFR) in REDCap. Entry into the eCRF will be verified with source data. A file defining location of all source data will be included in the TMF.

### 7.3.2 Handling of data

The Data Protection Regulation is complied with by archiving and shipping biological material. All documents related to the investigation will be retained for a period of at least 5 years after trial termination. Personal information from the participating subjects is dealt with in accordance to Danish law concerning handling of personal information.

Data will be kept behind locked doors on a secure computer, or in paper form and archived until final analysis has ended. During the study, electronic data will be kept safely in a REDCap database, only accessible to internal project group members.

The sponsor-investigator agree to allow direct access to all source data including the participant's medical files, during monitoring, auditing, and/or inspection by an ethics committee.

# 8.0 Information from patient records

## 8.1 Information before patient consent

Prior to inclusion in the project, the patient is assessed clinically for the presence of type 1 diabetes mellitus and symptoms of gastrointestinal neuropathy. This information appear from the patient's electronic patient record and the severity of the diabetic neuropathy is assessed by previously described patient history and diagnostic tests on neuropathy. This information is necessary for recruitment as well as proper identification of eligible patients for the trial. Information from the patient records, which has been used before consent has been obtained, will be passed on to the project if the patient is included.

## 8.2 Information after patient consent

The specific health information, which is obtained by the treating physician from the patient's electronic patient's electronic patient record (Midt-EPJ) and passed on to the project is important for the clinical assessment and follow-up of the patient. Information is documented on an ongoing basis in the patients record and includes:

a) Personal information (gender, age, previous and current treatments in the health systems
b) Clinical information (medical records of clinical status, comorbidity, diarrhea, constipation, abdominal pain, vomiting, treatment decisions, medical history)
c) Microbiological test results (History of bacteria, viruses, fungi and parasites in faeces, blood and other secretions)
d) Blood test results (C-reactive protein, leukocytes, albumin, kidney and fluid counts, hemoglobin, glucose and HbA1c)
e) Medication list (current treatment and previous antibiotics)
f) Other examinations (image diagnostics, colonoscopy)

Consent to inclusion in the research project gives the person responsible for the trial, the sponsor, the sponsor's representatives and any control authorities direct access to obtain the information in the patient's medical record. The purpose of this it to extract data, control purposes and monitoring. These are necessary for the implementation of the project. Patient data are obtained on an ongoing basis by inclusion and clinical attendance.

# 9.0 Personal information

Personal information will be stored safely in www.REDCap.au.dk and processed lawfully. The project will be carried out in accordance with the Danish Data Protection Act and the European General Data Protection Regulation. Only data necessary for investigation of the study objectives will be gathered, and inaccurate data will be corrected or deleted. Participants can at any time withdraw their informed consent and no further data will be collected if they object it. All data will be deleted or anonymised no later than 10 years after the trial is terminated. The project will be registered at the Central Denmark Region directory.

# 10.0 Financial statement

The project group took initiative to the trial and is responsible for the idea and design of the study. AUH (Bloodbank/Pharmacy) provides FMT and placebo capsules and is involved in recruitment of donors and preparation of faeces. Steno Diabetes center fund the study expenses, including PhD salaries (Table 2). Salary payments go through AU, who pays the PhD student's salaries. Steno Diabetes center will be invoiced for administrative and operational costs from AUH (Account holder: Central Denmark Region, Viborg). There are no other financial connections.

**Table 2. Budget**

| | **From Steno Diabetes center** |
|---|---|
| **Salaries** | 1.548.000 DKK |
| **Administrative and operational cost** | 1.356.721 DKK |
| **Total** | 2.904.721 DKK |

# 11.0 Reimbursement

Participation involves nine visits at AUH. The visits only require that the participants stay at AUH for a few hours at a time. All participants can be reimbursed for documented transport expenses and lost wages after each completed intervention period. Reimbursement for lost wages is considered taxable as B-income.

# 12.0 Recruitment and informed consent

Participants will be recruited by informing potentially eligible outpatients in contact with the Department of Hepatology and Gastroenterology at AUH about the trial. The patients can be notified when arriving at the department for a scheduled appointment or by generating a list of patients with the diagnose code E10.7 at the department. These patients may then receive a letter in their e-Boks with contact information on the contact person in the study. If a patient shows interest in the trial, a meeting with the contact person will be scheduled. The
patient is informed about the possibility of bringing a bystander, and the meeting will take place in a secluded room at AUH. The patient will receive written and oral information about the project, have time to ask questions, and will be offered 48 hours to consider participation. The writ-

ten information will include the amendment: *"Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt"*. Following informed consent, a screening assessment may determine if the patient meets the Inclusion criteria. If so, the patient can be included in the trial. If not, the patient is listed as a screen failure in the screening log.

# 13.0 Publication of study results

We will register the trial in **www.clinicaltrials.org**, and send a clinical study report to the National Patient Safety Agency. During the execution of the project, major changes will be reported. We will attempt publication of both positive, negative as well as inconclusive results in relevant national and international peer-reviewed journals. The first author will be appointed according to the Vancouver system. At the end of the project, all trail patients will receive a trail summary, and patients can be informed by individual request whether they received treatment with FMT or placebo.

# 14.0 Ethical considerations

The trial will be performed according to the Helsinki Declaration (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). The investigator will at all times let the consideration for the individual patient's overall treatment outweigh the consideration for the project. Participation is voluntary, and enrollment will only occur following written and oral information and documentation of informed consent. If a patient chooses not to participate, or at any time to withdraw their informed consent, it will not influence further investigations or treatments at the hospital.

Possible side effects are considered to be mild and short lasting. The risk of side effects is acceptable considering the potential advantages from the results of this study, which may produce new knowledge about the gut functions and microbiota in patients with DM1 and severe gastrointestinal neuropathy. When the necessary precautions are taken, it is our professional assessment that the gain from the treatment significantly exceeds the risk and disadvantage. For these reasons, we consider it responsible to conduct the study.

# 15.0 Insurance

In case of injuries caused by study participation, the law of patients' insurance, the law of medical injury, and the law of product responsibility insure the participants.

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**Figure 1**. Diagram of study visits and investigations

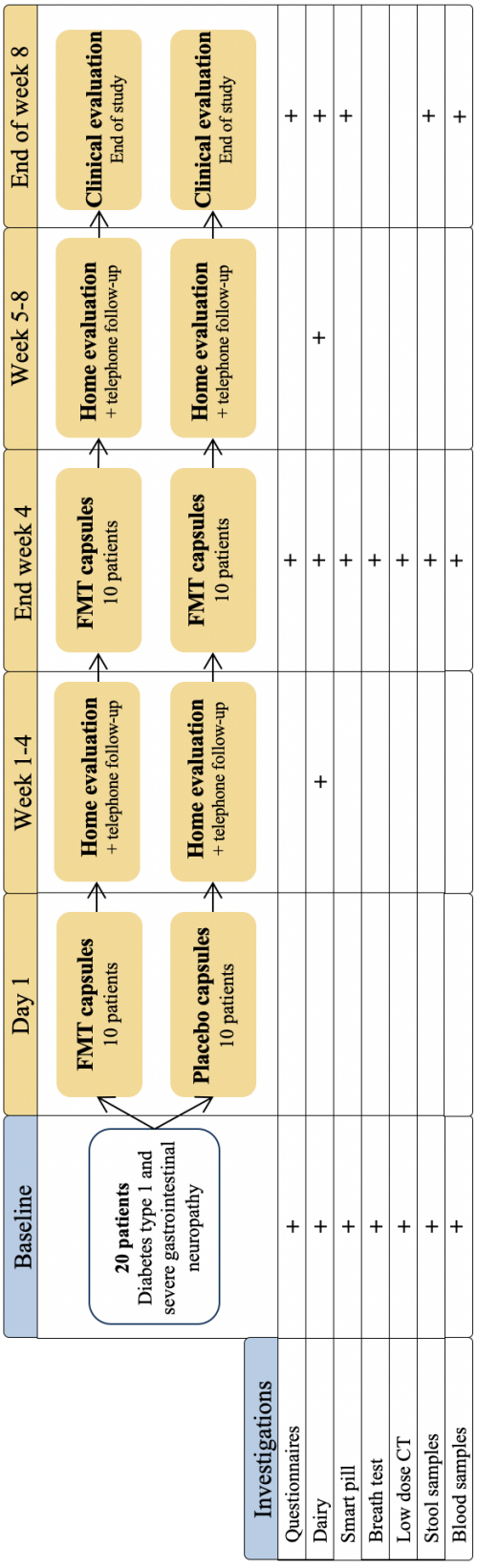

**Figure 2.** Diagram of study flow.

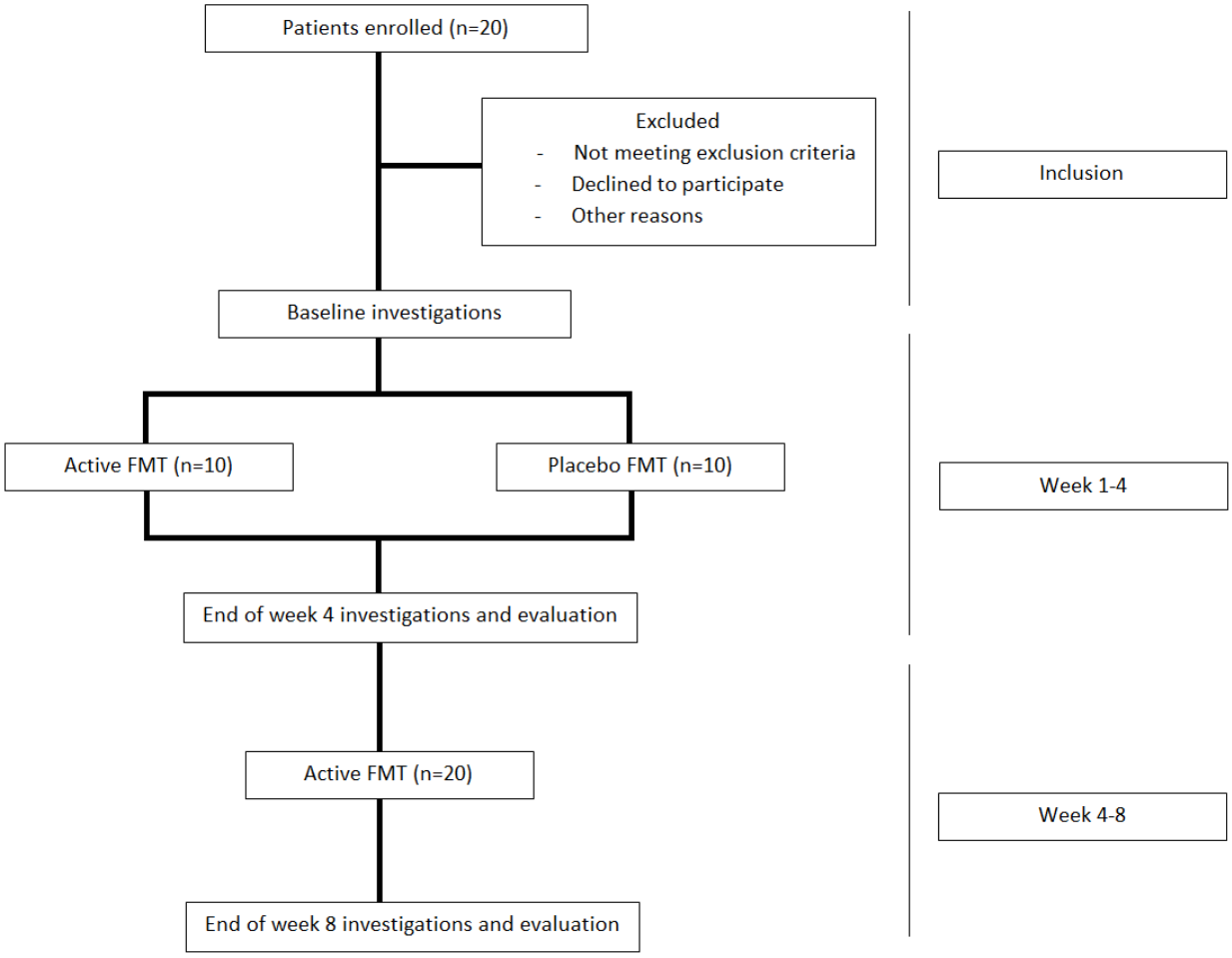

**Figure 3.** Tracing the WMC illustrating gastric emptying (GET), small bowel transit time (SBTT) and colorectal transit time (CTT). The drop in pH (green graph) is clearly seen as the capsule passes from the small intestine to the caecum.