Statistical analysis plan (SAP)
Project title: Faecal Microbiota Transplantation against chronic diarrhoea in Patients with Systemic Sclerosis - a randomised, double-blinded, safety and pilot-efficacy study
Version 07, 02 March 2026

NCT06333795

AIM: This study aims to evaluate the feasibility, safety, and pilot efficacy of capsule-based faecal microbiota transplantation (FMT) as a treatment against chronic diarrhoea in patients with Systemic Sclerosis.

Unblinding and principles

This Statistical Analysis Plan (SAP) provides details on the analysis and presentation of results for our clinical trial Faemicue. The SAP is finalised before any statistical analysis. The statistical analysis will be performed when the last patient has completed all visits in the trial, and all data have been entered into the REDCap database. All data will be extracted from the electronic CRF stored in the REDCap database hosted by Aarhus University. All analyses will be performed in R or STATA Version 19.5 (STATA Corp, Texas, USA).

1) Sponsor will write the laboratory in C104 and request the document, which contains the codes for which patients received placebo and which patients received FMT.
2) The randomisation document will be handed to the project manager, who will then divide the patients into two groups named "treatment 1 " and "treatment 2".
3) An additional investigator, Jesper W Andersen, who is not a project investigator and remains blinded to which treatment belongs to which group, will then perform the analysis.

Statistical analysis of the primary and secondary endpoints will be performed according to the intention-to-treat principle; thus, all randomised patients are included in their initially assigned study arm, regardless of compliance with the study protocol. All randomised patients are expected to complete the study.

Patient demographics, including gender, age, BMI, duration of systemic sclerosis (SSc), and antibody status, will be summarised and compared between treatment groups using descriptive statistics. The variables for the two groups will be compared using Chi-square or Fisher's exact tests, and continuous variables using Student's t-test or Wilcoxon rank-sum test, as applicable.

Primary endpoint

Primary endpoint, from protocol: Number of adverse events (AE) severity grade 2 or more, assessed by CTCAE v5.0. during the first week after intervention (FMT or placebo). Adverse events (AEs) will be classified and graded according to the **Common Terminology Criteria for Adverse Events (CTCAE) v5.0**.

1. Study primary endpoint: Number of patients with adverse events (AE) severity grade 2 or more, assessed by CTCAE v5.0. during the first week after intervention (FMT or placebo).

The association between the FMT group compared to the placebo group will be presented as a Relative Risk (RR). The RR is computed by dividing the risk of adverse events of severity grade 2 or more in the FMT group with the risk of adverse events of severity grade 2 or more in the placebo group. The RR will be presented with 95% confidence intervals (95% CI). The primary binary outcome will be tested for significance with a Chi-square test (if all expected values 5 or higher) or Fisher's exact test (if any expected value is below 5). A P-value < 0.05 will be considered statistically significant.

Secondary endpoints

Safety-related secondary endpoints:

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

The association between the number of patients with adverse events of severity grade 2 or more assessed by the CICAE v5.0 in the FMT group compared to the placebo group will be assessed with a mixed-effect model, with the patient as the random effect.

Descriptive statistics will be used to summarise adverse events (AEs), including the number and percentage of participants experiencing each AE, stratified by severity (Grade 1, Grade 2, Grade 3, etc.). All unique types of AE will be described in total numbers and percentages to investigate the commonness of symptoms. The number of reactions to the AE or SAE will also be reported. SAE's will also be reported in numbers and percentages. We will also describe the number of AE, whether there are more AE registered at the first treatment compared to the last treatment.

Patient-reported outcome measures are obtained from the questionnaires and the bowel habit diaries completed at baseline, one week after each treatment and 4 weeks after each intervention period. The treatment effect will be assessed by comparing changes in score between the two groups, from baseline to weeks 5 and 9 and between weeks 5 and 9, using Student's t-test or a non-parametric test, depending on whether the data follow the normal distribution or not. A P-value < 0.05 will be considered statistically significant.

3. Number of bowel movements within a week.
4. Median stool consistency (Bristol Stool scale)
5. Number of passages of hard stools (BS1-2)
6. Number of passages of liquid stools (BS6-7)
7. Median number of bowel movements per 24 hours
8. Number of nightly bowel movements (from bedtime until morning)
9. Number of episodes with involuntary defecation
10. Average of symptom complaints during the intervention weeks.

11. Mild adverse events (grade 1) following FMT, or placebo, assessed by CTCAE v5.0
12. Adverse events in week 1-4 in each intervention period
13. Serious adverse events during all 26 weeks of the study.
14. Difference in Likert scale for Bowel symptoms between active FMT and placebo
15. Differences in the Likert scale for Bowel symptoms between 1 or 2 components as the initial treatment dose.
16. Change in Gastrointestinal symptoms – through questionnaire UCLA SCTC GIT 2.0 score.
17. Change in patient assessment of Faecal incontinence (Wexner faecal incontinence score)
18. Change in irritable bowel syndrome impact scale (GSRS-IBS)
19. Change in Quality of Life (ED5Q-5L)
20. Severity of symptoms and impact on daily life each week on a scale from 1-5. One being the lowest and 5 being the most severe.

Objective outcome measures from the low-dose CT scan and breath test performed at baseline and 4 weeks after the first intervention period will provide information on:

21. Volume of the colon
22. Changes in the rise of hydrogen and methane measured in the breath test performed

Microbiome assessment comes from faecal samples collected at baseline, week 1, week 5 and week 9. We will investigate the following hypothesis:

23. Treatment with FMT changes the colonic microbiota in patients with SSc, compared to those who received placebo treatment. A standard bioinformatic pipeline will be applied following DNA extraction and whole genome sequencing: taxonomic profiling including alpha diversity, beta diversity, observed taxa.

Sponsor-investigator date and signature

2-3-2026

Klaus Krogh
Professor, overlæge, dr.med.
regionmidtjylland
Lever-, Mave- og Tarmsygdomme
Aarhus Universitetshospital
Palle Juul-Jensens Boulevard 99 • 8200 Aarhus N
E-mail: klaukrog@rm.dk

Principal investigator date and signature

2-3-2026

Klaus Krogh
Professor, overlæge, dr.med.
regionmidtjylland
Lever-, Mave- og Tarmsygdomme
Aarhus Universitetshospital
Palle Juul-Jensens Boulevard 99 • 8200 Aarhus N
E-mail: klaukrog@rm.dk