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The FLORA trial

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Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised placebo-controlled trial

The FLORA trial

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ABSTRACT

Introduction: An unbalanced intestinal microbiota may mediate activation of the inflammatory pathways seen in psoriatic arthritis (PsA). A randomised, placebo-controlled trial of faecal microbiota transplantation (FMT) infused into the small intestine of PsA patients with active peripheral disease who are non-responsive to methotrexate (MTX) treatment will be conducted. The objective is to explore clinical aspects associated with FMT performed in PsA patients.

Methods and analysis: The FLORA trial is a randomised, two-centre stratified, double-blind (patient, care provider and outcome assessor), placebo-controlled, parallel-group study. Eighty patients will be included and randomised (1:1) to either placebo (saline) or FMT provided from an anonymous healthy donor. Throughout the study, both groups will continue the weekly self-administered subcutaneous (s.c.) MTX treatment, remaining on the pre-inclusion dosage (15-25 mg/week). The clinical measures of psoriasis and PsA disease activity used include the Health Assessment Questionnaire (2-page DI-HAQ), the Dermatology Quality of Life Index (DLQI), the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index, the Psoriasis Area Severity Index (PASI), a dactylitis digit count, a swollen/tender joint count (66/68), plasma C-reactive protein as well as visual analogue scales for pain, fatigue, and patient and physician global assessments. The primary endpoint is the proportion of patients who experience a treatment failure during the 6-month trial period. The number of adverse events will be registered throughout the study.

Ethics and dissemination: This is a proof-of-concept clinical trial and will be performed in agreement with God Clinical Practice standards. Approvals have been obtained from the local Ethics Committee (DK-S-20150080) and the Danish Data Protection Agency (15/41684). The study has commenced in May 2017. Dissemination will be through presentations at national and international conferences and through publications in international peer-reviewed journals.

Trial registration number: NCT03058900

Strengths and limitations of this study

- This is a double-blind, randomised, placebo-controlled trial of faecal microbiota transplantation in psoriatic arthritis (PsA).
- Subcutaneously administered MTX treatment.
- The primary endpoint is based on shared decision-making between patient and physician.
- Associated microbiome analyses can reveal novel insight into the PsA pathogenesis.
- A limitation of the study is that the content of the faecal transplant suspension cannot be fully standardized.

INTRODUCTION

Emerging data suggest a causal relationship between the intestinal microbiota and spondyloarthritis (SpA), thus, linking dysbiosis of the complex microbial communities with SpA pathogenesis.[1-5] Psoriatic arthritis (PsA) is one of five SpA categories in adults which also include ankylosing spondylitis, undifferentiated arthritis, reactive arthritis and arthritis associated with inflammatory bowel disease. While the association between the gut and the latter two disorders is well established,[6] only very recently, studies evaluating the faecal microbiota and the presence of subclinical gut inflammation in PsA patients have coupled this disease to a perturbation of the intestinal microbiota composition.[7-12]

PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum and a varied disease course.[13] The clinical manifestations include peripheral arthritis, enthesitis and/or spondylitis combined with more or less severe psoriatic skin involvement, nail psoriasis, and dactylitis.[14] Nearly half of the patients with both early and established PsA also present with extra-musculoskeletal manifestations which can include bowel (16%), ocular, cardiovascular or urogenital involvement.[15] Without disease modifying intervention, 40-60% of PsA patients will develop erosive and deforming joint damage within a few years of disease onset.[16] Methotrexate (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug (csDMARD) for initial therapy.[17] However, a substantial number of patients does not benefit from such treatment.[18] Currently, other treatment options may include biological agents such as tumour necrosis factor (TNF-α) inhibitors aiming to block some of the downstream molecular pathways driving the disease.[19] Howbeit, these drugs do not target the cause of PsA, which is believed to be multifactorial comprising genetic, immunological and environmental factors.[20] The interplay between these complex aetiological factors has yet to be fully understood.[21,22]

The classic pathophysiological concept of PsA is that it is an autoimmune disease of the skin and joints and that the pathological processes at both sites are driven by inflammatory responses involving the innate immune system, natural killer cells, T cells, and the expression of pro-inflammatory cytokines, including TNF-α, interleukin (IL)-1, interferon-γ, IL-6, IL-12, IL-15, IL-18 and the IL-17/IL-23 axis.[23-27] However, although microbial agents including dormant bacteria, bacterial products, mycobacteria and viral antigens have been implicated as potential initiators,[28,29] the true pathophysiological factors triggering the dysregulated immunological cascade underlying the disease remain to be identified.

Intriguingly, it has recently been suggested that mucosal sites exposed to a high load of bacterial antigens, in particular the gut, may represent the initial site of immunological tolerance break in PsA.[30] Indeed, under normal conditions the host and the microbiota live in harmony and benefit from their mutualistic relationship. However, alterations of the normal intestinal microbiota can affect mucosal immunity which, in turn, can induce local inflammation and elicit systemic effects at distant sites.[31] Mechanisms through which the intestinal microbiota may be involved in the pathogenesis of PsA include an abnormal activation of the gut-associated lymphoid tissue,[32] a decrease in regulatory T cell activity,[33] and/or an altered mucosal permeability thus compromising the capacity of the intestine to provide adequate containment of luminal microorganisms and molecules.[34,35] In support of these theories, several studies have documented subclinical gut inflammation in PsA patients.[36-41] Moreover, a recent study reported that several intestinal bacteria including *Akkermansia* and *Ruminococcus* were practically absent in PsA

patients. These commensal bacteria are, in fact, known to play an important role in maintaining gut homeostasis.[42]

Rationale

If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen in PsA,[8] modifying the intestinal microbiota could be a novel treatment strategy for this disease.[1-3,43] Faecal microbiota transplantation (FMT) is currently being used to restore the balance of the intestinal flora.[44,45] Particularly, this procedure has demonstrated more than 90% clinical resolution of recurrent or refractory *Clostridium difficile* infections.[46-50] Also, multiple FMTs seem to be able to induce remission in patients with inflammatory bowel disease (IBD).[51] Due to these results, FMT is now being tested as a potential novel treatment for other gastrointestinal and extra-intestinal diseases.[52] To the best of our knowledge, no study has yet ascertained the efficacy and safety of FMT in patients with inflammatory rheumatic diseases.

Evidence-based research

To avoid waste of research no new studies should be initiated without a systematic review of the existing evidence.[53] We performed a pragmatic search in the biomedical literature via Pubmed combining different related MeSH terms: ("Microbiota"[Mesh] OR "Fecal Microbiota Transplantation"[Mesh] OR "Faecal Microbiota Transplantation"[Mesh] OR "Gastrointestinal Microbiome"[Mesh]) AND (arthritis[tiab] OR "Arthritis"[Mesh] OR "Arthritis, Psoriatic"[Mesh] OR “Arthritis, Reactive”[Mesh] OR “Spondylarthritis”[Mesh] OR "Arthritis, Gouty"[Mesh] OR "Arthritis, Rheumatoid"[Mesh] OR "Psoriasis"[Mesh]). From the search revealing 122 citations it became clear that the majority of papers were reviews/editorials (74 [61%]), where the overall conclusion was that the main challenges are to uncover the cause-effect relationship between the intestinal microbiota and rheumatic diseases, and to investigate the potential of microbiome-targeting strategies.[1,3,5,6,20,32,43,54-60] Also from the published literature it became evident that to date only three clinical interventional studies trying to modify the intestinal microbiota in arthritis patients have been performed; one in enthesis-related arthritis using probiotics (n = 8),[61] one in juvenile idiopathic arthritis using exclusive enteral nutrition (n = 7),[62] and one in rheumatoid arthritis patients using probiotics in a placebo-controlled setting (n = 60).[63] Following the intervention, the latter two studies showed a moderate anti-inflammatory effect on the number of active joints, on the Disease Activity Score of 28 joints (DAS-28), and on the C-reactive protein concentrations. In the first study reporting no beneficial effects, the probiotics did not change the microbiota. No clinical trials performing FMT on arthritic patients were identified.

Objective

By conducting a double-blind, randomised, placebo-controlled trial, the objective of this study is to explore whether FMT is more effective than placebo in reducing disease activity in PsA patients with active peripheral arthritis concomitantly treated with weekly subcutaneously administered MTX. In addition, extensive bacteria taxonomic and metagenomic analyses will be performed on faecal samples before and after the FMT to get an indication of the functional capacity of the intestinal microbiota.

METHODS AND ANALYSIS

Trial design

This is a randomised – patient, physician and outcome-assessor blinded, placebo-controlled, 6-month trial, which will be followed by an open-label extension trial for a minimum of 2 years. Patients will be randomly assigned in a 1:1 ratio to receive FMT or placebo (sham procedure). Outcome assessment will be based on follow-up by a rheumatologist and is scheduled to occur after 3 and 6 months (primary end-point evaluation), see Figure 1 and Figure 2.

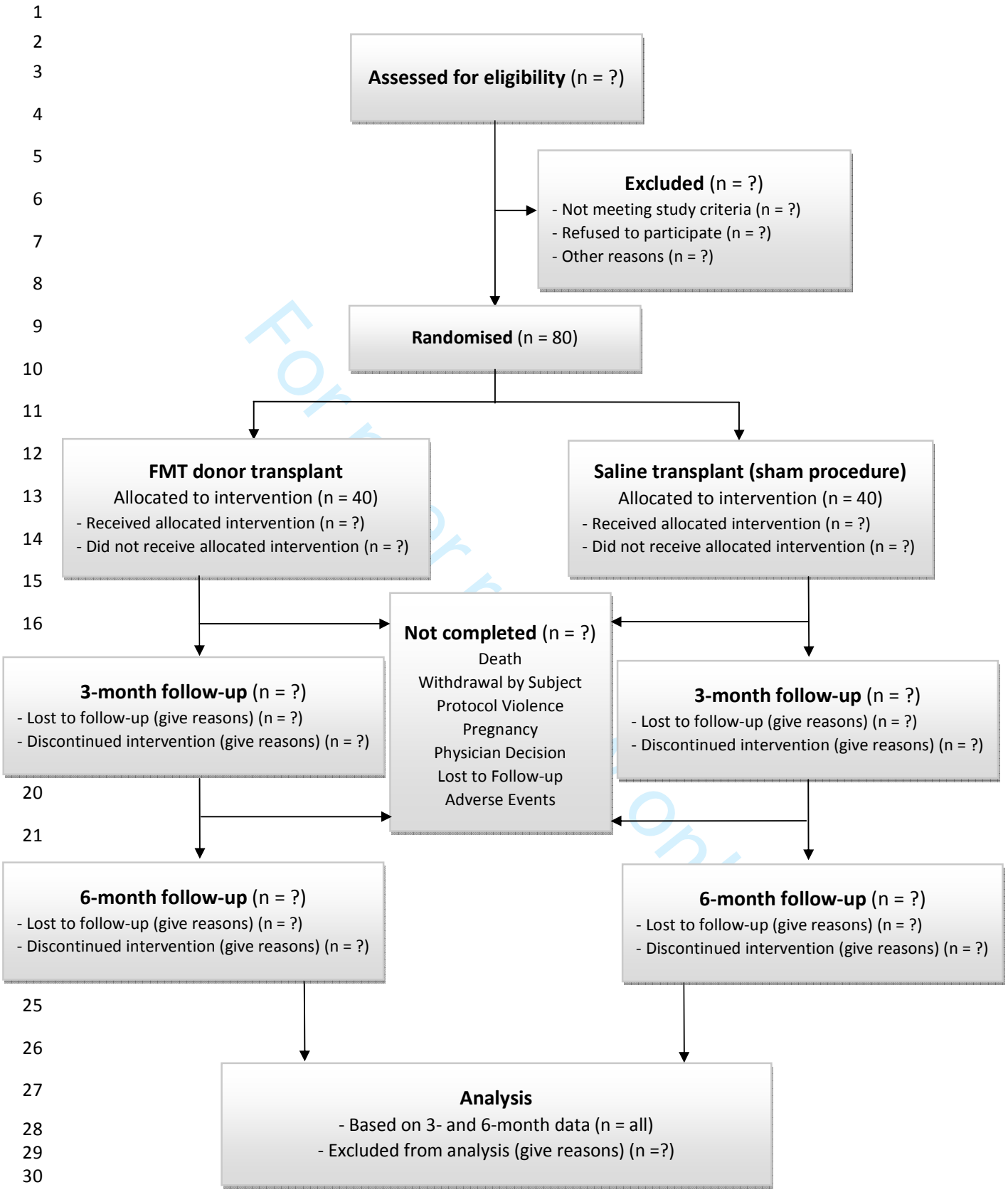

Figure 1. Flow diagram of the randomised, placebo-controlled trial

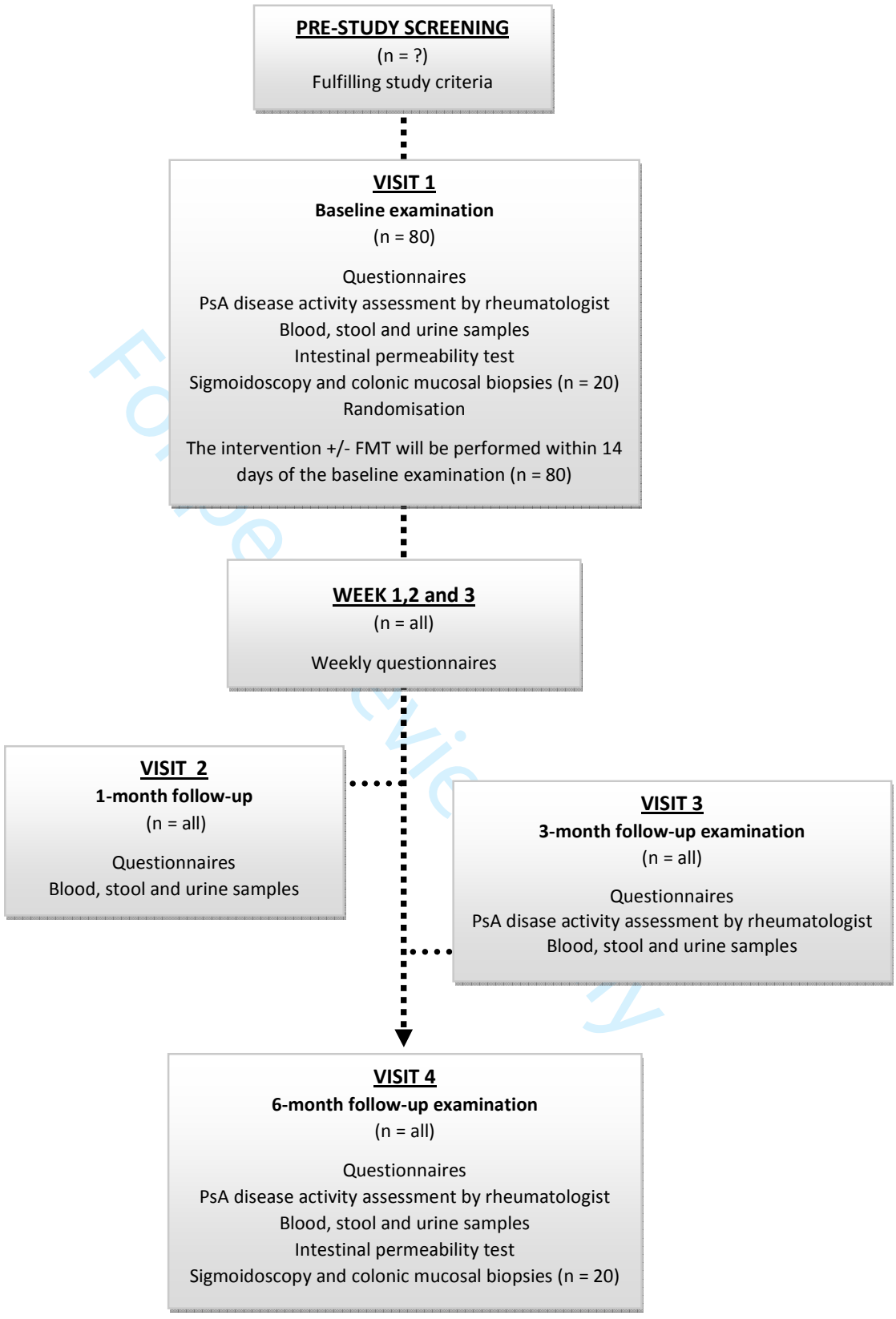

Figure 2. Participation timeline and general characteristics of each visit.

Participants

Patients fulfilling the inclusion criteria will be offered participation. No treatment with biologics within 6 months, and no systemic and/or local intra-articular or peritendinous steroid injections, or non-MTX csDMARD treatment, or antibiotics are allowed within 3 months of inclusion. Non Steroidal Anti-Inflammatory Drugs (NSAIDs) must be paused within 14 days of study inclusion, and throughout the 6-month follow-up period. Patients, who do not wish to participate, will be characterised by sex and age. The recruitment has commenced in May 2017 and will continue until 2019.

Psoriatic arthritis patients

A total of 80 PsA patients will be enrolled, and they will have to meet the following criteria:

Inclusion criteria:

- Diagnosis of PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR).[64]
- Presence of active peripheral arthritis defined as ≥ 3 swollen joints.
- Subcutaneously administered MTX treatment (≥ 15mg/week (maximal tolerable dosage)) for a minimum of 3 months prior to study inclusion.
- Age 18 to 70 years.

Exclusion criteria:

- Other rheumatic inflammatory diseases than PsA.
- Clinical suspicion of current axial disease activity.
- History of severe MTX toxicity or allergic reactions.
- Biological treatment within the last 6 months.
- Non-MTX DMARD treatment within 3 months of inclusion.
- Systemic and/or local intra-articular or peritendinous steroid injections within 3 months of inclusion.
- NSAIDs within fourteen days.
- Antibiotics within 3 months of inclusion.
- Inflammatory bowel disease, celiac disease, food allergy, or other intestinal diseases.
- Pregnant or breastfeeding women.
- Not wishing to participate or unsuited for project evaluation.

Stool donors

The stool donor corps will consist of three to five anonymous (to the recipient) donors who must be healthy as assessed by a screening questionnaire, and be active members of the Danish blood donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m^2, and an average alcohol intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior

to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie diets), and the donor must not be in a stressful life period. Before joining the stool donor corps, each potential donor will go through a screening process including stool analyses for faecal calprotectin and enteric pathogens (*Aeromonas, Campylobacter, C. difficile*, diarrhoeagenic *Escherichia coli, Salmonella, Shigella, Vibrio, Yersinia enterocolitica,* and multidrug-resistant bacteria, parasites including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA), *Cryptosporidium* (DNA) and Giardia (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus (RNA), human adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood tests for C-reactive protein (CRP), white blood cell count, haemoglobin, albumin, alanine aminotransferase (ALAT), glomerular filtration rate (eGFR) and coeliac disease, and blood test for infectious agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus (IgM), hepatitis A, B, C and E, tuberculosis (QuantiFERON® TB-Gold test), syphilis, human immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies), and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing the screening tests, the donor will donate stool for the next month after which, the donor will have to pass the screening programme once more before the stool can be released for transplantation.

Interventions

Overall study interventions

The FMT will be an add-on strategy for PsA patients with active joint disease despite ongoing treatment with weekly subcutaneously administered MTX. Therefore, all enrolled PsA patients will continue their MTX treatment throughout the study, and they will remain on the same individual dosage that they received at the time of study inclusion (a minimum of 15 mg/week cf. the patient inclusion criteria) in addition to folic acid supplement. Paracetamol and tramadol in recommended dosages are allowed during the trial but no NSAIDs can be taken.

Active and sham comparator

Patients will be randomised into two groups with an allocation ratio of active-to-placebo treatment of 1:1. The active comparator group (n = 40) will have an FMT with healthy donor faeces-suspension (250 mL) containing 50 g donor faeces, saline (NaCl 0.9%) and glycerol (10%), whereas, the sham comparator group (n = 40) will be treated with an identical appearing sham procedure where the transplant solution will consist of 250 mL brown coloured (brown food colourant) isotonic saline (NaCl 0.9%).

Preparing the FMT suspension

Donors will collect faeces at home and transport it in a cooling bag to the study site within 1 hour. Faeces will be sieved to remove particulate material, followed by dilution in sterile saline (0.9% NaCl) and 10% glycerol. The FMT suspension will be stored at - 80 ^{o}C until use. On the day of the FMT transplantation, the transplant suspension (250 mL) will be thawed to 37 ^{o}C and subsequently apportioned into five 50 mL syringes.

FMT procedure

The FMT will take place within 14 days (preferably 7 days) of the baseline clinical examination. The evening prior to the FMT, patients will take one dose of oral proton-pump inhibitor. They will meet at the Department of Gastroenterology after a six-hour fast. A total of 250 mL transplant suspension (active or placebo) will be installed in the duodenum using an oral-duodenal tube. The correct placement of the tube will be confirmed using gastroscopic guidance.

Treatment strategy for FMT non-responders

Patients who present with increased disease activity during follow-up will, depending on the clinical presentation, be offered another treatment strategy which may include local intra-articular steroid injections, change to another csDMARD or biological treatment. If the patient accept such treatment changes, this will be characterised as FMT treatment failure according to the primary outcome definition (one intra-articular steroid injection is allowed).

MTX toxicity and drop-outs

Blood tests for MTX toxicity will be performed in accordance with our current clinical practice. In case of MTX toxicity, severe side effects, pregnancy, or occurrence of infectious disease or other diseases that contraindicate MTX treatment, MTX dosage will be decreased or the treatment will be paused. These patients will remain in the study (unless their condition contraindicates this), and they will be analysed as members of the treatment group to which they were randomised using intention-to-treat-type analyses.

Outcomes

Primary Outcome Measure:

Treatment failure [Time Frame: 6 months (+/- 14 days)]

Proportion of patients in each group who experience treatment failure according to shared decision making between patient and rheumatologist defined as at least one of the following:

- Need for more than one intra-articular glucocorticoid injection due to disease activity.
- Need for change to other csDMARDs (e.g., oral leflunomide or sulfasalazin) according to the updated Danish guideline treatment due to disease activity.
- Need for biologic treatment according to the updated Danish treatment guideline due to severe disease activity.

Secondary Outcome Measures:

Change from baseline in the Short Health Assessment Questionnaire (2-page HAQ)[65,66] [Time Frame: Baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire[67] [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Changes from baseline in patient reported gastrointestinal side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Other non-gastrointestinal patient reported side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Proportion of patients in each group achieving the American College of Rheumatology (ACR)[68] Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

I. ACR20 response criteria[69]

II. ACR50 response criteria[70]

III. ACR70 response criteria[70]

Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)[68] [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index[68] in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Psoriasis Area Severity Index (PASI)[71] in the subset of patients who have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the number of digits affected with dactylitis in the subset of patients who have dactylitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]

Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14 days)]

Tertiary (exploratory secondary) outcomes: Proportion of patients in each group achieving changes in plasma CRP, changes in tender point count,[72] changes in faecal bacteria composition and metabolism, changes in intestinal permeability,[73] changes in plasma orosomucoid, changes in plasma and faecal calprotectin,[74] changes in serum 1,25-dihydroxyvitamin D, changes in cardiovascular risk factors including Body Mass Index (BMI), blood pressure, plasma triglyceride, plasma LDL-cholesterol, plasma HDL-cholesterol, plasma total-cholesterol, and HbA_1C levels,

changes in specific circulating inflammatory markers (i.e. cytokines, adipokines, and chemokines), and macroscopic and microscopic inflammatory changes of the colonic mucosa, see Table 1.

Safety

The most frequently reported adverse events (AEs) related to FMT are vomiting, belching, mild diarrhoea, abdominal cramping, transient fever and elevated C-reactive protein on the day of the procedure.[75] A recent systematic review on the adverse events of FMT identified 50 relevant studies with a total of 1,089 patients. In this review, the incidences of serious adverse events (SAEs) for FMT were 2.0% and 6.1% for upper and lower gastrointestinal routes, respectively. The SAEs that probably or possibly were related to FMT included infections (0.7%), IBD flare (0.6%), death (0.3%), auto-immune diseases and FMT procedure related injury.[76] Although most of the patients included in this review suffered from severe gastrointestinal diseases (*C. difficile* infection and/or IBD), these findings warrant caution when performing FMT; especially when introducing the procedure in a new patient population. In addition, the potential long term side effects following FMT remains largely unknown.[77] Still, when strict donor screening is conducted and the procedure is performed by experienced practitioners, FMT is in general considered safe, and even elderly patients with a poor medical condition and multiple co morbidities as well as immunosuppressed patients have been proven to tolerate the FMT procedure well.[78-82]

In the present study, we will carefully monitor and evaluate safety by means of open assessment of AEs. All reported or observed AEs are recorded by the investigators, and will be monitored until resolution, stabilisation or until it has been shown that the study intervention is not the cause. The National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 (NIH publication # 09-7473), will be used to grade the severity of adverse events. Gastrointestinal side effects (nausea, vomiting, abdominal pain, number of stools pr. week, stool type (Bristol Stool Chart), blood or mucus in the stool) will be registered by the patients once a week for the first month following the randomised intervention. Routine blood screening for MTX toxicity will normally be performed at week 4, 10, 16, 22 but can be more frequent if decided by the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Subject incidence rates of all treatment-emergent AE will be tabulated by system organ class and preferred term. Tables of fatal AEs, SAEs, AEs leading to withdrawal from study, and significant treatment-emergent adverse events, will also be provided. For the long-term extension portion of this study, exposure adjusted event rates will be summarised.

Sample size and power considerations

For a comparison of two independent binomial proportions using the Pearson's chi-squared statistic with a Chi-square approximation (a two-sided significance level of 0.05), a sample size of 40 PsA patients per group has a power of 90% (0.895) if we assume that the proportions of treatment failures are 35% (FMT-active group) and 70% (FMT-sham group), respectively. Consequently, the inclusion of 80 PsA patients allocated (1:1) to two treatment arms is believed to be sufficient to reveal any difference of clinical importance between treatment groups (i.e., an

NNT <3 patients). Data will be analysed with the STATA statistical package (version 15; StataCorp LP), and SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA).

Assuming that there will be some attrition during the 6-month trial period, we also estimated how much drop out would be possible while still having a reasonable statistical power (80%): a total sample size of 62 PsA patients assuming a comparable level of withdrawals (31 patients completing in each group) achieves a power of at least 0.8 with the proportion of treatment failures indicated above; i.e., even if we experience a drop-out rate of 20%, our trial will have 80% chance of detecting the intentional difference between groups

Randomisation, allocation concealment and blinding

The randomisation was conducted using central-computer randomisation. Patients will be randomly allocated (1:1) to receive either a FMT or a placebo saline transplant (sham procedure). The randomisation lists was generated by the trial statistician and uploaded to the REDCap database by an independent data manager who will not be involved in any other aspects of the trial. Eligible patients will - after signing informed consent - be assigned randomly in permuted blocks with varying sizes of 4 and 6, according to computer-generated random numbers (SAS programming via SAS PROC PLAN), to undergo either FMT or a saline (sham) procedure using stratification for centre. The randomisation of each patient will be implemented by the local trial coordinator and allocation will be concealed as this is done independent of the pre-determined sequence generation (i.e. randomisation). The patients, care providers and outcome assessors will remain unaware of the group assignments, and only de-identified codes will be used to link participants to their data during the study to maintain their confidentiality.

Data collection, management and confidentiality

Data will be entered via a secure web-based electronic clinical report form (eCRF) into a central REDCap[83] database hosted by Odense Patient data Explorative Network (OPEN) at Odense University Hospital. Data obtained during the clinical examination will be entered directly into the database. Also, patient questionnaires will be fulfilled directly into the database. Access to the study data will be restricted, and a password system will be utilized to control access. All information about the patients' health and other private matters is covered by confidentiality. The authorisation from the Danish Data Protection Agency has been secured.

Statistical methods

The full analysis set will consist of all randomised participants (i.e., the Intention to treat population). Participants will be analysed according to their randomised treatment group. Descriptive statistics will be provided for demographics, and baseline characteristics. The summary statistics of continuous variables will include: N, mean, standard deviation, median, interquartiles, and range. All summaries presenting frequencies and incidences will include counts, % and N, where N is the total number of participants in the corresponding arm.

The pre-specified efficacy analyses will be based data from the full-analysis set, which include all patients who underwent randomisation, have had their baseline measurement performed, and who have received the initial transplant (independent of group). The safety analysis set will include all patients who were randomly assigned to a study group and had exposure to a transplant (independent of group). Missing values will be imputed with the of a non-responder imputation by use of the baseline-observation-carried-forward method for measurements made after baseline. Thus, missing data for dichotomous endpoints will also be imputed using a “null responder” imputation, assuming that the patient did not have any benefit from being enrolled in the trial (e.g., for the primary endpoint will assume that the patient had a treatment failure).

Categorical changes for dichotomous end points will be analysed with the use of logistic regression with the model including treatment and centre as class effects. For continuous outcome measures an analysis of covariance (ANCOVA) model will be used to analyse mean changes in continuous end points. The model will include treatment, centre, with the baseline value of the relevant variable as a covariate. Sensitivity analyses, will be performed to assess the robustness of the primary analyses, including “worst” and “best” case imputation, repeated-measures and multiple-imputation analyses, using model-based approaches; repeated measures linear mixed models will also be used to model the potential group-dependent trajectories over time.

Additionally, completer analyses will be performed on those who complete 6 months of treatment. During follow-up, any medical treatments which could potentially modify the intestinal microbiota including antibiotics will be reported, but will not affect the statistical analysis. Statistical estimates will be calculated as odds ratios (OR) for the dichotomous variables and difference between means for continuous outcomes reported with 95% confidence intervals (95% CI). Two-sided confidence intervals, and P-values for primary, secondary and exploratory outcomes will be computed and will not be adjusted for multiplicity.

Exploratory stratified analyses will investigate whether the treatment effect varies with I) the faecal microbiota analysis performed at follow-up compared with baseline (+/- long-term changes in the intestinal microbiota and intestinal inflammation); and II) the demographic match (sex, age) between the stool donor and the recipient. Non-responders will represent the outcome group not fulfilling the primary outcome measure. Differences in demographics and baseline disease activity between this treatment-failure subpopulation and the remaining group will be examined in order to identify potential predictors for poor responders. Patients not participating in the follow-up examination will be classified as "drop-outs", and if possible, the reason for not participating will be registered.

Activity/assessment	Pre-study screening	Visit 1 Baseline	Week 1, 2 and 3	Visit 2 1 month	Visit 3 3 months	Visit 4 6 months
Patients	n = ?	n = 80	n = all	n = all	n = all	n = all
Screening log	x					
Inclusion/exclusion form	x					
Consent form		x				
Randomisation		x				
Study-composed questionnaire		x	x	x	x	x
Patient global (VAS 0-100 mm)		x	x	x	x	x
Patient fatigue (VAS 0-100 mm)		x	x	x	x	x
Patient pain (VAS 0-100 mm)		x	x	x	x	x
HAQ		x	x	x	x	x
BASDAI		x			x	x
BASFAI		x			x	x
DLQI		x	x	x	x	x
Gastrointestinal symptom diary		x	x	x	x	x
Eating habits questionnaire		x				
Clinical examination:						
- Height (m)		x				
- Weight (kg)		x			x	x
- Blood pressure (mmHg)		x			x	x
- Psoriasis Area Severity Index		x			x	x
- SPARCC Enthesitis Score		x			x	x
- Swollen joint count (66)		x			x	x
- Tender joint count (68)		x			x	x
- Doctors global (VAS 0-100 mm)		x			x	x
- BASMI		x			x	x
- Tender point count		x			x	x
Interview (AEs)				x	x	x
Blood sample analysis:						
- C-reactive protein (mg/L)		x		x	x	x
- Orosomucoid (g/L)		x		x	x	x
- Calprotectin		x		x	x	x
- 1,25-dihydroxyvitamin D (nmol/L)		x		x	x	x
- TSH (miu/L)		x				x
- Hgb (mmol/L)		x				x
- Triglyceride (mmol/L)		x				x
- LDL-cholesterol (mmol/L)		x				x
- HDL-cholesterol (mmol/L)		x				x
- Total-cholesterol (mmol/L)		x				x
- HbA_1C (mmol/mol)		x				x
- HLA-B27 status (+/-)		x				
- Serology tests for *Yersinia, Campylobacter*, *Salmonella* (+/-)		x				
Faecal calprotectin		x		x	x	x
Faecal microbiota analysis		x		x	x	x
Sigmoidoscopy and mucosa biopsy		x				x
Stool, blood, and urine samples (biobank)		x		x	x	x
Intestinal permeability test		x				x
Intervention (+/- FMT)		x				
Serious adverse event forms				x		

Table 1. Protocol schedule of forms and procedures

ETHICS AND DISSEMINATION

This study is designed as a proof-of-concept clinical trial and will be performed in agreement with GCP-standards, and in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration (64th, 2013). The relevance of the study, the design and the recruitment strategy were evaluated with three patient research partners (PRPs), and alterations especially in primary outcome and recruitment strategy were embedded. Furthermore, a minimum of two PRPs (participating in the study) will be involved in the discussion regarding the progress of the recruitment phase and results, and will be offered the opportunity to comment on the manuscript draft. The Regional Committees on Health Research Ethics for Southern Denmark (DK-S-20150080) and the Danish Data Protection Agency (15/41684) have approved the study protocol, and the trial has been registered with ClinicalTrials.gov (NCT03058900). The Danish Health and Medicines Authority does not classify the FMT procedure as a medical intervention, and has had no objection to the use of FMT for this study and patient category. Thus, no GCP auditing is legally required. A report describing any potential side effects and adverse events will be submitted to the Ethics Committee yearly.

Although the Danish Health Authorities, for the time being, do not classify donor faecal microbiota as tissue, all steps of the stool donor recruitment, stool donation and FMT preparation will be in accordance with the Danish Tissue Law to ensure that the quality and safety standards laid down in the Danish Legislation BEK nr 764 of May 26, 2015 (implementing Directive 2004/23/EC) are met. Three to five stool donors will be recruited from the South Danish Transfusion Service & Tissue Center, Department of Clinical Immunology, Odense University Hospital, and they will be carefully screened for potentially transmissible infections and other conditions associated with gut microbiota function before their stool can be released for FMT. Being a stool donor is voluntary, and no compensation fee will be given. Furthermore, to ensure donor traceability, each patient in the active treatment arm will only receive microbiota from one donor. Also, frozen samples will be clearly labeled with a unique donation code based on the ISBT 128 coding and labeling system, and the release of the final product will adhere to the standards for tissue and blood donation.

Due to the well-documented risk of permanent joint destruction and occurrence of extra-articular manifestations in the PsA disease course, identification of new treatment modalities and biomarkers is essential to help the physician to slow down the disease development or ultimately to prevent it. All PsA patients participating in this study have significant activity in their joint disease despite treatment with the current guideline treatment and first-line drug, MTX, for this condition. This patient population will therefore benefit greatly from new treatment options. Consequently, when weighing the pros and cons of this study, this trial should be performed from a scientific and ethical perspective.

Dissemination will occur through presentations at national and international conferences and publications in international peer-reviewed journal(s).

DISCUSSION

Recent years have seen growing recognition of the complexity of the role of the microbiota in shaping the immune system and its potential effects for health and disease.[22,84,85] In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.[86-89] Intriguingly, an abnormal intestinal bacteria composition has been observed in PsA patients, and this association has fostered theories linking intestinal dysbiosis and PsA joint inflammation.[90] Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic disease are causal related,[55] and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.[54,91] We expect that this double-blind, randomised, placebo-controlled trial will shed new light on this highly relevant topic.

This is the first time that the efficacy and safety of FMT is being investigated in MTX immunosuppressed patients with rheumatic diseases. Subsequently, no data on the feasibility of conducting FMTs in the rheumatological setting is, so far, available. Nor do we know whether one FMT will be sufficient, or whether it should be repeated shortly after the first intervention to normalise the alterations of the intestinal microbiota, which would expectedly enhance any potential anti-inflammatory effects. In the present proof-of concept clinical trial, the FMT procedure is considered an add-on to the current guideline intervention and first-line drug, MTX. Therefore, from a pragmatic and ethical perspective, we have decided to perform only one FMT (or sham procedure) in each patient even if we are well aware that this approach may not be adequate to achieve long-lasting effects. Indeed, in patients with inflammatory bowel diseases it appears that performing a frequent dosing-regime repeating the FMT procedure up to five times a week for eight weeks provided the best results.[51,92,93] Hence, in contrast to the treatment of *C. difficile* infections where the microbiota is pushed past the point of homeostasis and can be restored following only one FMT,[47] the chronic nature of PsA and other autoimmune and inflammatory diseases, and the somewhat lesser degree of intestinal dysbiosis, may make the host microbiota more resistant to long-lasting modifications. Nevertheless, we strongly believe that the FMT procedure in the present study will be sufficient to boost the effects of MTX so that the participants who are all MTX-non-responders prior to study enrolment will achieve disease control without needing to add or switch to other non-MTX medication.

In the present trial, the primary endpoint is defined as the occurrence of treatment failure according to shared decision making between patient and physician evaluated at 6 months following the randomised intervention (FMT versus sham procedure). Shared decision making is a process in which both patient and health professional make a decision, taking into account the best evidence of available treatment options and the patient's values and preferences. This approach is considered a key element in the management of rheumatic diseases.[94] In addition to the primary end point evaluation at 6 months, patients will be asked to fill out a weekly questionnaire regarding side effects as well as skin and arthritis symptoms during the first month following the randomised intervention to reveal any short-term effects on patient-reported outcomes.

Next, only patients with active peripheral PsA will be included. One reason for this is that this will be the first time that FMT is performed on rheumatic patients. Therefore, it seems

reasonable only to enrol patients who have not had adequate effect from the initial guideline treatment (MTX), and consequently, on an individual basis could benefit the most from participating in new experimental clinical trials. Also, since patients need to have at least three swollen joints, we expect that we will be able to detect treatment effects of clinical importance. The fact that we do not include recent onset treatment naive patients will, of course, limit our ability to generalise our findings unto the entire PsA patient population. Indeed, in a recent randomised controlled trial of FMT in ulcerative colitis patients, participants with a recent diagnosis (< 1 year) were statistically significantly more likely to respond to FMT compared with those with longer disease duration.[92] That patients will have to subcutaneously administer MTX for at least three months prior to study enrolment will ensure that low intestinal MTX absorption is excluded as a potential effect modifier for the poor MTX response. In addition, as many drugs, including MTX, seem to affect the intestinal microbiological millieu,[95-98] bypassing the intestine during MTX administration will ensure that no local non-disease related effects on the intestinal microbiota will occur.

A great challenge when conducting a trial of FMT is that for the present being there is a lack of both national and international recommendations guiding the regulation and the best clinical practices for donor screening, stool sample handling and preparation of the FMT suspension.[99-101] Indeed, the variability in faecal bacterial communities can complicate or undermine treatment efficacy. This variability stems from both biological variation and variation introduced by sample handling. A recent study reported that oxygen exposure degraded faecal bacterial communities, whereas freeze-thaw cycles and lag time between donor defecation and transplant preparation had much more limited effects.[102] Given that many intestinal bacteria are obligate anaerobe, including many beneficial bacteria potentially possessing anti-inflammatory effects, exposure to oxygen during the preparation of FMT may potentially compromise the therapeutic value of FMT in PsA and other inflammatory diseases. Therefore, although frozen faecal preparations of stool suspended into physiological saline and glycerol have proven just as effective as fresh stool in treating *C. difficile* infections,[103] the optimal transplant preparation method in treating inflammatory diseases remain to be established.

Our stool handling setup is in line with the prevailing practice, which includes mixing and filtration of the stool suspension and adding saline and glycerol as a cryopreservative before storage at minus 80 ºC.[101] In addition, we have sought to limit the oxygen exposure during transport by placing the donor stool within a plastic bag, which is subsequently put into a tightly closed small plastic container. Supplementary, during preparation the solution will not be homogenized for more than 10 seconds. Nevertheless, the lack of optimal anaerobe conditions during stool handling could possibly undermine the therapeutic potential of our FMT procedure. Furthermore, although we aim to use 50g of faeces for each transplant, we acknowledge that the exact weight between donations could vary with an estimated +/- 5 g. Also, due to the wide variability in microbial content in stool between donations, the content cannot be fully standardized, and may likely differ between each FMT procedure. However, to meet this challenge we will collect and store samples from each donation which will enable us to determine the microbiota composition of each donation in case some donations prove more effective than others.

Stool donor selection is another important issue that needs to be addressed. The composition of the normal microbiota composition has only recently been mapped,[104] and the existence of a limited number of well-balanced host–microbial symbiotic states, where one or more bacteria species are considered the main functional driver(s), have been identified using clustering of metagenomic sequences.[105] Still, the most favourable donor microbiota composition for treating inflammatory diseases has yet to be determined. Therefore, it also remains to be established whether donors with a high stool bacteria diversity should be preferred over isolation of specific bacteria, or if pooled stool samples from several donors outperforms a single-donor transplant.[51,106] We have chosen to use only single donations from three to five different anonymous stool donors to ensure donor traceability and to enable us to identify any individual donor-specific microbial effects. Also, since host intrinsic-, environmental-, dietary- and medication factors have been associated with gut bacteria composition and functionality,[95,96,107,108] the donors must eat a balanced diet, not be overweight or take any medications or be physical or psychological stressed, smoke or consume alcohol during the donation period in order to limit the risk of transferring "abnormal" microbiota to the recipients. These donor criteria have been set for safety reasons, and we acknowledge, that this could potentially limit the inter-donor microbiota diversity due to shared lifestyle characteristics.

Another factor to keep in mind is the concept of matching donor and recipient, which may be of importance for enhancing the colonisation capabilities of the donor microbial communities. In fact, Rossen et al[93] did find that in patients with ulcerative colitis, the microbiota of FMT responders shifted to their respective donors, whereas non-responders did not. Li et al[109] reported that donor bacteria strains established extensively in the recipient and persisted for at least 3 months with a negligible decline of donor-strain populations detected between 45 days and 3 months following FMT in metabolic syndrome patients. However, they also found that recipients receiving the same donor transplant displayed varying degrees of microbiota transfer, indicating individual patterns of microbiome resistance and donor-recipient compatibilities. In addition, host genetics is known to effect the gut microbiota,[110] and animal models have shown that sex[111] and age[112] also can be potentially modifiers of the gut bacteria composition. These observations may prove to be of importance for the outcome of FMT in inflammatory diseases.[113] However, whether sex- and/or age-matching between donor and recipient is crucial for a successful FMT in humans remains to be enlighten. Therefore, in the present study, no donor-recipient matching will be conducted. However, a subgroup analysis will be performed to reveal any trend that could indicate better results in sex- or age-match cases.

Furthermore, as the interactions between the microbiota and the host are influenced by cooperation and competition between pathogenic and commensal microbes and multiple environmental variables, the lifestyle of the recipient following the FMT may be of importance. Nevertheless, due to the uncertainty of how to define the optimal lifestyle and the lack of knowledge on how different lifestyle factors may interfere with the microbiota, we have decided that the patients in the present study will not have to adhere to any predefined lifestyle "regime" or diet following the randomised intervention. However, every participant will fulfil an eating habit questionnaire at the beginning of the trial.

Finally, non-bacteria microorganisms such as bacteriophages, viruses and fungi may also be of importance when targeting components of the microbiota or host cells for therapeutic purposes.[114-116] Other complicating factors may include the composition of other microbiological niches such as the oral, lung, genitourinary, and skin microbiota.[117,118] Indeed, the latter could likely prove to be of significance in patients with skin psoriasis. However, these factors will not be assessed in the present study.

CONCLUSION

Autoimmune and inflammatory rheumatic diseases are characterised by an abnormal gut bacteria composition. This trial has the potential to substantially expand the growing body of literature on the role of the intestinal microbiota in PsA, thereby enhancing our understanding of cause and effect. The results of this study, when completed, may be exploited for biomarker discovery, and for diagnostic and therapeutic purposes.

AUTHORS' CONTRIBUTION

T. Ellingsen, M.S. Kragsnaes, R. Christensen, and J. Kjeldsen conceived and developed the idea for the study. T. Ellingsen and M.S. Kragsnaes are the principal investigators and wrote the first study protocol draft. T. Ellingsen and M.S. Kragsnaes were responsible for all communication with the scientific ethical committee, the Danish Data Protection Agency and the National Health Board. T Ellingsen is the responsible party and sponsor. M.S. Kragsnaes, T. Ellingsen, H.C. Horn, J.K. Pedersen, H.L. Munk, and U. Fredberg sat up the inclusion and exclusion criteria for the psoriatic arthritis patients, and the latter five rheumatologists are conducting all the clinical examinations. J. Kjeldsen, F.M. Pedersen, and H. Glerup discussed and planned the FMT procedure, and they are conducting the FMTs and the sigmoidoscopies (obtaining mucosae tissue samples). D.K. Holm and H.M. Holt helped set up the donor screening programme, and they were responsible for conducting this programme and performing the microbiological and immunological tests. V. Andersen and K. Kristiansen are responsible for the microbiome and omics analyses, and have advised on how the tissue collection should be performed and what kind of tissue would be relevant to collect. R. Christensen has written the analysis plan and will be responsible for the final statistics analyses. In conclusion, all participants designated as authors have contributed to the conception and design of the study, and have critically either drafted or revised the first draft of the study protocol and the protocol paper. Also, all authors have approved the final version before submission.

REGISTRATION

The trial has been registered with ClinicalTrials.gov (NCT03058900).

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COMPETETING INTEREST STATEMENT

None of the team members of this research project has declared any potential conflict of interest.

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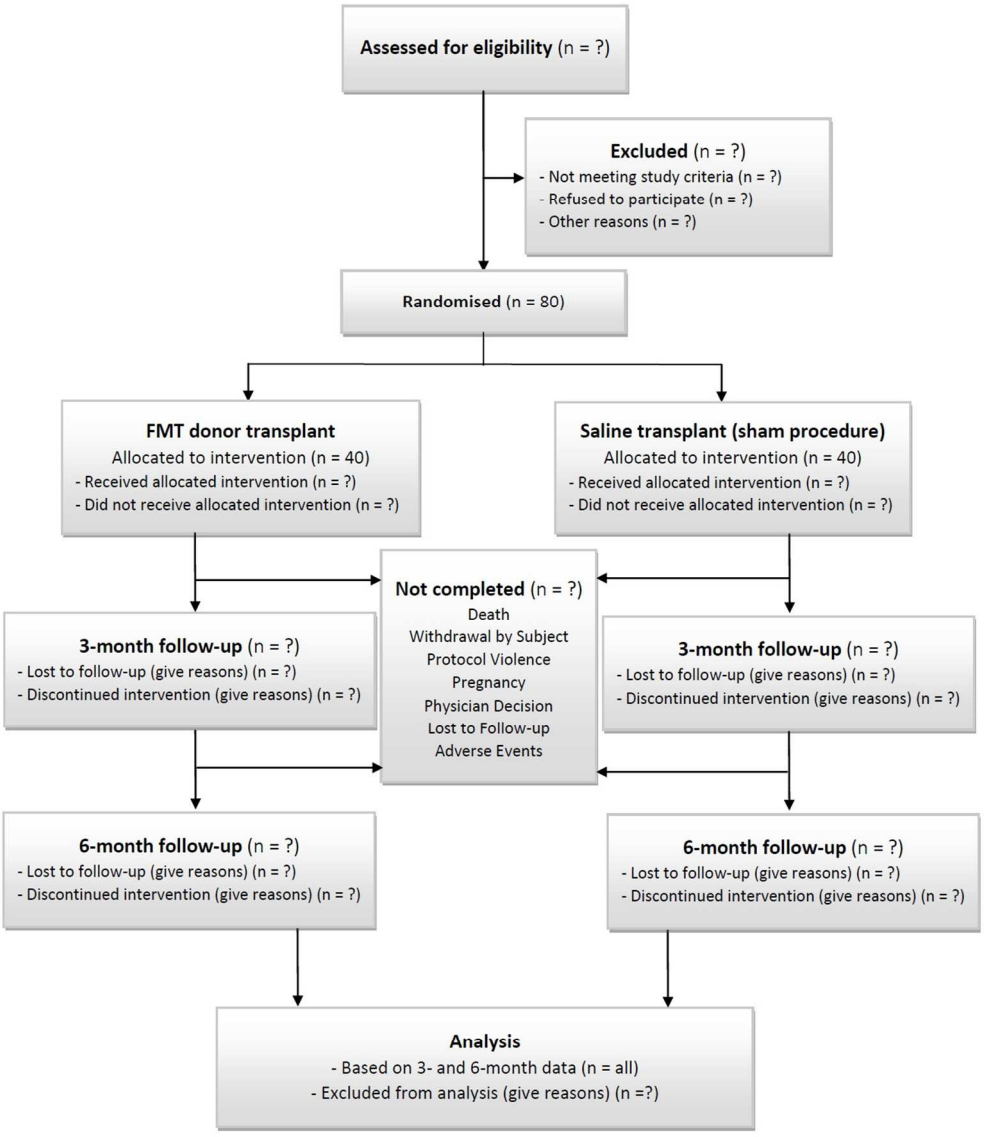

Figure. 1. Flow diagram of the randomised, placebo-controlled trial

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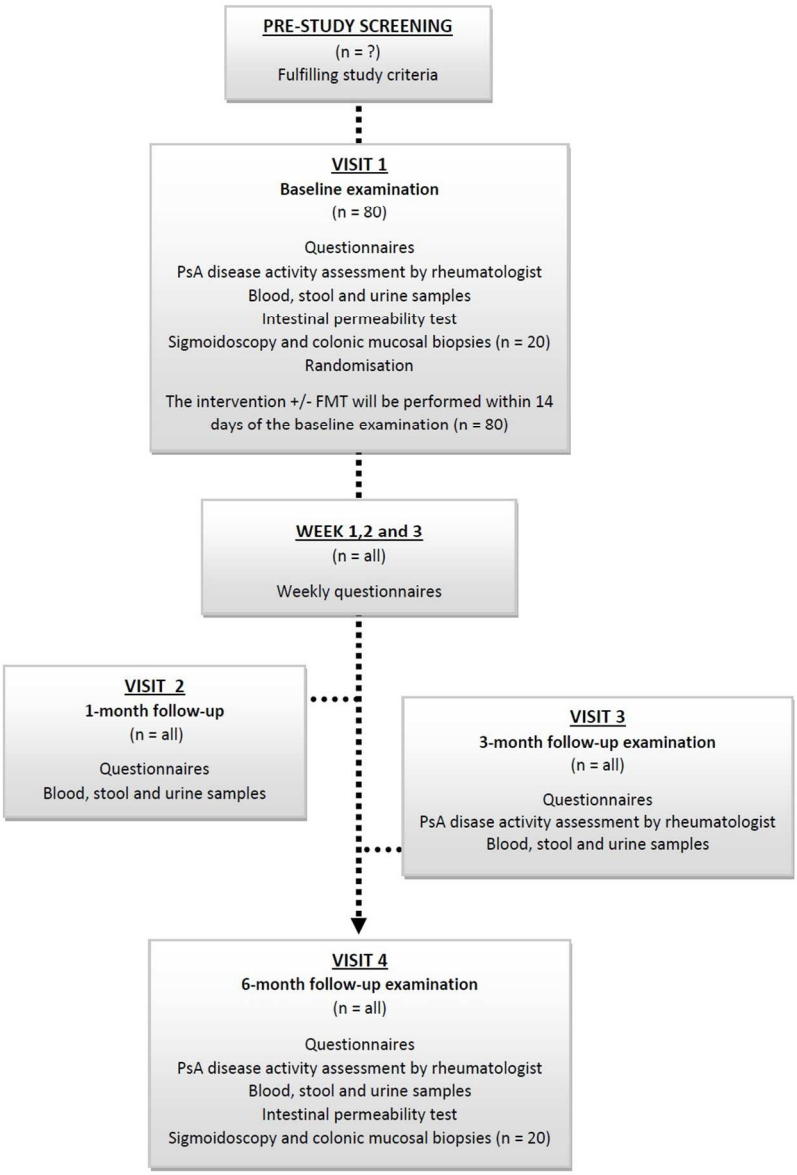

Figure 2. Participation timeline and general characteristics of each visit

151x203mm (192 x 192 DPI)

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HLP/bss

Forskningsprojekt:
Fæces-mikrobiom-transplantation hos patienter med perifer psoriasisgigt: Et 6-måneders randomiseret, placebo-kontrolleret studie. Eudract nr.: ?

Den Videnskabsetiske Komité for Region Syddanmark har på sit møde den 17. juni 2015 behandlet ovennævnte forskningsprojekt og truffet følgende:

Afgørelse
Komiteen har godkendt projektet på vilkår i henhold til lov nr. 593 af 14. juni 2011 om videnskabsetisk behandling af sundhedsvidenskabelige forskningsprojekter

279x179mm (192 x 192 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	8-9
	4b	Settings and locations where the data were collected	
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	9-10
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	
	6b	Any changes to trial outcomes after the trial commenced, with reasons	10-11
Sample size	7a	How sample size was determined	12-13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	13
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	13
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	13
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	13
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	13

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	9
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	13-14
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	14
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	6
	13b	For each group, losses and exclusions after randomisation, together with reasons	6
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	-
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	-
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	-
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	-
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	12
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	17-20
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	17-18
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	-
Other information			
Registration	23	Registration number and name of trial registry	20
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	20

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised placebo-controlled trial

The FLORA trial

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Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised placebo-controlled trial

The FLORA trial

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ABSTRACT

Introduction: An unbalanced intestinal microbiota may mediate activation of the inflammatory pathways seen in psoriatic arthritis (PsA). A randomised, placebo-controlled trial of faecal microbiota transplantation (FMT) infused into the small intestine of PsA patients with active peripheral disease who are non-responsive to methotrexate (MTX) treatment will be conducted. The objective is to explore clinical aspects associated with FMT performed in PsA patients.

Methods and analysis: The FLORA trial is a randomised, two-centre stratified, double-blind (patient, care provider and outcome assessor), placebo-controlled, parallel-group study. Eighty patients will be included and randomised (1:1) to either placebo (saline) or FMT provided from an anonymous healthy donor. Throughout the study, both groups will continue the weekly self-administered subcutaneous (s.c.) MTX treatment, remaining on the pre-inclusion dosage (15-25 mg/week). The clinical measures of psoriasis and PsA disease activity used include the Health Assessment Questionnaire (2-page DI-HAQ), the Dermatology Quality of Life Index (DLQI), the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index, the Psoriasis Area Severity Index (PASI), a dactylitis digit count, a swollen/tender joint count (66/68), plasma C-reactive protein as well as visual analogue scales for pain, fatigue, and patient and physician global assessments. The primary endpoint is the proportion of patients who experience treatment failure during the 6-month trial period. The number of adverse events will be registered throughout the study.

Ethics and dissemination: This is a proof-of-concept clinical trial and will be performed in agreement with Good Clinical Practice (GCP) standards. Approvals have been obtained from the local Ethics Committee (DK-S-20150080) and the Danish Data Protection Agency (15/41684). The study has commenced in May 2017. Dissemination will be through presentations at national and international conferences and through publications in international peer-reviewed journal(s).

Trial registration number at ClinicalTrials.gov: NCT03058900

Strengths and limitations of this study

- This is a double-blind, randomised, placebo-controlled trial.
- Subcutaneously administered MTX treatment.
- The primary endpoint is based on shared decision-making between patient and physician.
- No feasibility data regarding FMT in rheumatic patients were available when the trial was designed.
- A limitation of the study is that the content of the faecal transplant suspension cannot be fully standardised.

INTRODUCTION

Emerging data suggest a causal relationship between the intestinal microbiota and spondyloarthritis (SpA), thus, linking dysbiosis of the complex microbial communities with SpA pathogenesis.[1-5] Psoriatic arthritis (PsA) is one of five SpA categories in adults which also include ankylosing spondylitis, undifferentiated arthritis, reactive arthritis and arthritis associated with inflammatory bowel disease. While the association between the gut and the latter two disorders is well established,[6] only very recently, studies evaluating the faecal microbiota and the presence of subclinical gut inflammation in PsA patients have coupled this disease to a perturbation of the intestinal microbiota composition.[7-12]

PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum and a varied disease course.[13] The clinical manifestations include peripheral arthritis, enthesitis and/or spondylitis combined with more or less severe psoriatic skin involvement, nail psoriasis, and dactylitis.[14] Nearly half of the patients with both early and established PsA also present with extra-musculoskeletal manifestations which can include bowel (16%), ocular, cardiovascular or urogenital involvement.[15] Without disease modifying intervention, 40-60% of PsA patients will develop erosive and deforming joint damage within a few years of disease onset.[16] Methotrexate (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug (csDMARD) for initial therapy.[17] However, the evidence for MTX in PsA is poor, and a substantial number of patients does not benefit from such treatment.[18] Currently, other treatment options may include biological agents such as tumour necrosis factor (TNF-α) inhibitors aiming to block some of the downstream molecular pathways driving the disease.[19] Still, these drugs do not target the cause of PsA, which is believed to be multifactorial comprising genetic, immunological and environmental factors.[20] The interplay between these complex aetiological factors has yet to be fully understood.[21,22]

The classic pathophysiological concept of PsA is that it is an autoimmune disease of the skin and joints and that the pathological processes at both sites are driven by inflammatory responses involving the innate immune system, natural killer cells, T cells, and the expression of pro-inflammatory cytokines, including TNF-α, interleukin (IL)-1, interferon-γ, IL-6, IL-12, IL-15, IL-18 and the IL-17/IL-23 axis.[23-27] However, although microbial agents including dormant bacteria, mycobacteria, bacterial products and viral antigens have been implicated as potential initiators,[28,29] the true pathophysiological factors triggering the dysregulated immunological cascade underlying the disease remain to be identified.

Intriguingly, it has recently been suggested that mucosal sites exposed to a high load of bacterial antigens, in particular the gastrointestinal tract, may represent the initial site of immunological tolerance break in PsA.[30] Indeed, under normal conditions the host and the microbiota live in harmony and benefit from their mutualistic relationship. However, alterations of the normal intestinal microbiota can affect mucosal immunity which, in turn, can induce local inflammation and elicit systemic effects at distant sites.[31] Mechanisms through which the intestinal microbiota may be involved in the pathogenesis of PsA include an abnormal activation of the gut-associated lymphoid tissue,[32] a decrease in regulatory T cell activity,[33] and/or an altered mucosal permeability thus compromising the capacity of the intestine to provide adequate containment of luminal microorganisms and molecules.[34,35] In support of these theories, several studies have documented subclinical gut inflammation in PsA patients.[36-41] Moreover, a recent

study reported that several intestinal bacteria including *Akkermansia* and *Ruminococcus* were practically absent in PsA patients. These commensal bacteria are, in fact, known to play an important role in maintaining gut homeostasis.[42]

Rationale

If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen in PsA,[8] modifying the intestinal microbiota could be a novel treatment strategy for this disease.[1-3,43] Faecal microbiota transplantation (FMT) is currently being used to restore the balance of the intestinal flora.[44,45] Particularly, this procedure has demonstrated more than 90% clinical resolution of recurrent or refractory *Clostridium difficile* infections.[46-50] Also, multiple FMTs seem to be able to induce remission in patients with inflammatory bowel disease (IBD).[51] Due to these results, FMT is now being tested as a potential novel treatment for other gastrointestinal and extra-intestinal diseases.[52] To the best of our knowledge, no study has yet ascertained the efficacy and safety of FMT in patients with inflammatory rheumatic diseases.

Evidence-based research

To avoid waste of research no new studies should be initiated without a systematic review of the existing evidence.[53] We performed a pragmatic search in the biomedical literature via Pubmed combining different related MeSH terms: ("Microbiota"[Mesh] OR "Fecal Microbiota Transplantation"[Mesh] OR "Faecal Microbiota Transplantation"[Mesh] OR "Gastrointestinal Microbiome"[Mesh]) AND (arthritis[tiab] OR "Arthritis"[Mesh] OR "Arthritis, Psoriatic"[Mesh] OR “Arthritis, Reactive”[Mesh] OR “Spondylarthritis”[Mesh] OR "Arthritis, Gouty"[Mesh] OR "Arthritis, Rheumatoid"[Mesh] OR "Psoriasis"[Mesh]). From the search revealing 122 citations it became clear that the majority of papers were reviews/editorials (74 [61%]), where the overall conclusion was that the main challenges are to uncover the cause-effect relationship between the intestinal microbiota and rheumatic diseases, and to investigate the potential of microbiome-targeting strategies.[1,3,5,6,20,32,43,54-60] Also from the published literature it became evident that to date only nine clinical interventional studies trying to modify the intestinal microbiota in arthritis patients have been performed: One study in SpA patients (n = 63),[61] and one study in enthesis-related arthritis (n = 8) reported no beneficial effects of probiotic therapy,[62] whereas one study in juvenile idiopathic arthritis testing exclusive enteral nutrition administration (n = 7) found a moderate anti-inflammatory effect on active joints.[63] Five placebo-controlled trials of probiotic therapy in rheumatoid arthritis patients[64-68] (sample size between 26 and 60 patients) reported mixed results.[69] However, two of these studies demonstrated positive clinical effects of probiotic therapy which included improvement in HAQ-DI pain scale,[65] improvement in the Disease Activity Score of 28 joints (DAS-28), and improvement on the C-reactive protein concentrations.[66] No clinical trials performing FMT on arthritic patients were identified.

Objective

The objective of this randomised trial is to explore whether FMT is more effective than placebo in reducing disease activity in PsA patients with active peripheral arthritis concomitantly treated with weekly subcutaneously administered MTX. In addition, extensive bacteria taxonomic and

metagenomic analyses will be performed on faecal samples before and after the FMT to get an indication of the functional capacity of the intestinal microbiota.

METHODS AND ANALYSIS

Trial design

This is a randomised – patient, physician and outcome-assessor blinded, placebo-controlled, 6-month trial, which will be followed by an open-label extension period for a minimum of 2 years. Patients will be randomly assigned in a 1:1 ratio to receive FMT or placebo (sham procedure). Outcome assessment will be based on follow-up by a rheumatologist and is scheduled to occur after 3 and 6 months (with the latter being the primary end-point evaluation), see Figure 1 and Figure 2.

Participants

Recruitment will take place at Danish rheumatology outpatient clinics, and patients fulfilling the eligibility criteria will be offered participation. No treatment with biologics within 6 months, and no systemic and/or local intra-articular or peritendinous steroid injections, or non-MTX csDMARD treatment, or antibiotics are allowed within 3 months prior to inclusion. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) must be paused within 14 days of study inclusion. Patients, who do not wish to participate, will be characterised by sex and age. The recruitment has commenced in May 2017 and will continue until 2019.

Psoriatic arthritis patients

A total of 80 PsA patients will be enrolled, and they will have to meet the following eligibility criteria:

Inclusion criteria:

- Diagnosis of PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR).[70]
- Presence of active peripheral arthritis defined as ≥ 3 swollen joints.
- Subcutaneously administered MTX treatment (≥ 15mg/week (maximal tolerable dosage)) for a minimum of 3 months prior to study inclusion.
- Age 18 to 70 years.

Exclusion criteria:

- Other inflammatory rheumatic diseases than PsA.
- Current axial disease activity or severe peripheral joint activity demanding immediate change of treatment or contraindicating placebo treatment for 6 months.
- Inflammatory bowel disease, coeliac disease, food allergy, or other intestinal diseases.
- Current cancer or severe chronic infections.

- History of severe MTX toxicity or allergic reactions.
- Biological treatment within 6 months prior to inclusion.
- Non-MTX DMARD treatment within 3 months prior to inclusion.
- Systemic and/or local intra-articular or peritendinous steroid injections within 3 months prior to inclusion.
- NSAIDs within 14 days prior to inclusion.
- Antibiotics within 3 months prior to inclusion.
- Pregnant or breastfeeding women.
- Not wishing to participate or unsuited for project evaluation.

Stool donors

The stool donor corps will consist of four anonymous (to the recipient) donors who must be healthy as assessed by a screening questionnaire, and be active members of the Danish blood donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m^2, and an average alcohol intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie diets), and must not be in a stressful life period. Before joining the stool donor corps, each potential donor will go through a screening process including stool analyses for faecal calprotectin and enteric pathogens (*Aeromonas, Campylobacter, C. difficile*, diarrhoeagenic *Escherichia coli, Salmonella, Shigella, Vibrio, Yersinia enterocolitica,* and multidrug-resistant bacteria, parasites including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA), *Cryptosporidium* (DNA) and Giardia (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus (RNA), human adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood tests for C-reactive protein (CRP) (acceptable level: < 6.0 mg/L), white blood cell count (acceptable range: 3.50-8.80 10^9/L), haemoglobin (acceptable range: 8.3-10.5 mmol/L), albumin (acceptable range: 36-50 g/L), alanine aminotransferase (ALAT) (acceptable range: 10-70 U/L), glomerular filtration rate (eGFR) (acceptable level: > 59 mL/min), and coeliac disease, and blood test for infectious agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus (IgM), hepatitis A, B, C and E, tuberculosis (QuantiFERON® TB-Gold test), syphilis, human immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies), and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing the screening tests, the donor will donate stool for the next month after which, the donor will have to pass the screening programme once more before the stool can be released for transplantation.

Interventions

Overall study interventions

The FMT will be an add-on strategy for PsA patients with active joint disease despite ongoing treatment with weekly subcutaneously administered MTX. Therefore, all enrolled PsA patients will continue their MTX treatment throughout the study, and they will remain on the same individual dosage that they received at the time of study inclusion (a minimum of 15 mg/week cf. the patient inclusion criteria) in addition to folic acid supplement. Paracetamol and tramadol in recommended dosages are allowed during the trial but no NSAIDs can be taken.

Active and sham comparator

Patients will be randomised into two groups with an allocation ratio of active-to-placebo treatment of 1:1. The active comparator group (n = 40) will have an FMT with healthy donor faeces-suspension (250 mL) containing 50 g donor faeces, saline (NaCl 0.9%) and glycerol (10%), whereas, the sham comparator group (n = 40) will be treated with an identical appearing sham procedure where the transplant solution will consist of 250 mL brown coloured (brown food colourant) isotonic saline (NaCl 0.9%).

Preparing the FMT suspension

Donors will collect faeces at home and transport it in a cooling bag to the study site within 1 hour. Faeces will be sieved to remove particulate material, followed by dilution in sterile saline (0.9% NaCl) and 10% glycerol. The FMT suspension will be stored at - 80 ^{o}C until use. On the day of the FMT transplantation, the transplant suspension (250 mL) will be thawed to 37 ^{o}C and subsequently apportioned into five 50 mL syringes.

FMT procedure

The FMT will take place within 14 days (preferably 7 days) of the baseline clinical examination. The evening prior to the FMT, patients will take one dose (40 mg) of oral proton-pump inhibitor. They will meet at the Department of Gastroenterology after a six-hour fast. A total of 250 mL transplant suspension (active or placebo) will be installed in the duodenum using an oral-duodenal tube. The correct placement of the tube will be confirmed using gastroscopic guidance.

Treatment strategy for non-responders

Patients who present with increased or unacceptable disease activity during the 6-month trial period will, depending on the clinical presentation, be offered another treatment strategy which may include local intra-articular steroid injections, change to another csDMARD or biological treatment. If the patient accepts such treatment changes, this will be characterised as FMT treatment failure according to the primary outcome definition (one intra-articular steroid injection is allowed).

MTX toxicity and drop-outs

Blood tests for MTX toxicity will be performed in accordance with our current clinical practice. In case of MTX toxicity, severe side effects, pregnancy, or occurrence of infectious disease or other diseases that contraindicate MTX treatment, MTX dosage will be decreased or the treatment will be paused. These patients will remain in the study (unless their condition contraindicates this),

and they will be analysed as members of the treatment group to which they were randomised using intention-to-treat-type analyses.

Collection of faecal samples and metagenomics analysis

Fresh faecal samples will be collected by the patient at home using an EasySampler® stool collection kit within 24 hours prior to the study visit. Samples will be stored in the patient's freezer until transport to the study site. During transport, samples will be kept on ice in a cooling bag. Upon arrival to the study site, samples will immediately be transferred to the biobank and stored at -80°C. Bacterial DNA will be extracted from the faecal samples following established standard protocols including bead beating using a NucleoSpin soil kit (Macherey-Nagel, Germany) according to manufacturer's instructions. DNA will be sequenced using the BGISEQ-500 Platform which was recently benchmarked against the Illumina platforms showing excellent intra-platform reproducibility and less GC bias than observed using the Illumina platforms (Fang et al. Submitted for publication). The faecal metagenomics bioinformatics analyses will be performed using comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics species,[71,72] taxonomic annotation, and extensive functional analyses based on metagenomic species which provides a superior dataset compared to the conventional analyses based on the total gene pool.[73]

Intestinal permeability test

After an overnight fasting, patients will provide a urine sample before ingesting 100 mL water containing 10 g of lactulose and 5 g of D-mannitol. All the urine passed in the subsequent 5 hours will be collected into a 2 L plastic container containing 1 mL of chlorohexidine (20 mg/mL) as a preservative. After 3- and 5 hours, the volume of the urine will be measured and a small volume (10 mL) will be preserved and stored at -80°C until analysis. No food or drinking (except for water) will be allowed during the test.[74,75]

Outcomes

Primary outcome measure:

Treatment failure [Time Frame: 6 months (+/- 14 days)]

Proportion of patients in each group who experience treatment failure according to shared decision making between patient and rheumatologist defined as at least one of the following:

- Need for more than one intra-articular glucocorticoid injection due to disease activity.
- Need for change to other csDMARDs (e.g., oral leflunomide or sulfasalazin) according to the updated Danish treatment guideline due to disease activity.
- Need for biologic treatment according to the updated Danish treatment guideline due to severe disease activity.

Secondary outcome measures:

Change from baseline in the Short Health Assessment Questionnaire (2-page HAQ)[76,77] [Time Frame: Baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire[78] [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Changes from baseline in patient reported gastrointestinal side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Other non-gastrointestinal patient reported side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Proportion of patients in each group achieving the American College of Rheumatology (ACR)[79] Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

I. ACR20 response criteria[80]

II. ACR50 response criteria[81]

III. ACR70 response criteria[81]

Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)[79] [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index[68] in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Psoriasis Area Severity Index (PASI)[82] in the subset of patients who have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the number of digits affected with dactylitis in the subset of patients who have dactylitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]

Number of adverse events in each group leading to discontinuation [Time Frame: 6 months (+/- 14 days)]

Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14 days)]

Tertiary (exploratory secondary) outcomes: Proportion of patients in each group achieving changes in plasma CRP, changes in tender point count,[83] changes in faecal bacteria composition and metabolism, changes in intestinal permeability, changes in plasma orosomucoid, changes in plasma and faecal calprotectin,[84] changes in serum 1,25-dihydroxyvitamin D, changes in cardiovascular risk factors including Body Mass Index (BMI), blood pressure, plasma triglyceride, plasma LDL-cholesterol, plasma HDL-cholesterol, plasma total-cholesterol, and HbA_1C levels, changes in specific circulating inflammatory markers (i.e. cytokines, adipokines, and chemokines), and macroscopic and microscopic inflammatory changes of the colonic mucosa, see Table 1.

Safety

The most frequently reported adverse events (AEs) related to FMT are vomiting, belching, mild diarrhoea, abdominal cramping, transient fever and elevated C-reactive protein on the day of the procedure.[85] A recent systematic review on the adverse events of FMT identified 50 relevant studies with a total of 1,089 patients. In this review, the incidences of serious adverse events (SAEs) for FMT were 2.0% and 6.1% for upper and lower gastrointestinal routes, respectively. The SAEs that probably or possibly were related to FMT included infections (0.7%), IBD flare (0.6%), death (0.3%), auto-immune diseases and FMT procedure related injury.[86] Although most of the patients included in this review suffered from severe gastrointestinal diseases (*C. difficile* infection and/or IBD), these findings warrant caution when performing FMT; especially when introducing the procedure in a new patient population. In addition, the potential long term side effects following FMT remains largely unknown.[87] Still, when strict donor screening is conducted and the procedure is performed by experienced practitioners, FMT is in general considered safe, and even elderly patients with a poor medical condition and multiple comorbidities as well as immunosuppressed patients have been proven to tolerate the FMT procedure well.[88-92]

In the present study, we will carefully monitor and evaluate safety by means of open assessment of AEs. All reported or observed AEs are recorded by the investigators, and will be monitored until resolution, stabilisation or until it has been shown that the study intervention is not the cause. The National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 (NIH publication # 09-7473), will be used to grade the severity of adverse events. Gastrointestinal side effects (nausea, vomiting, abdominal pain, number of stools pr. week, stool type (Bristol Stool Chart), blood or mucus in the stool) will be registered by the patients once a week for the first month following the randomised intervention. Routine blood screening for MTX toxicity will normally be performed at week 4, 10, 16, 22 but can be more frequent if decided by the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Subject incidence rates of all treatment-emergent AE will be tabulated by system organ class and preferred term. Tables of fatal AEs, SAEs, AEs leading to withdrawal from study, and significant treatment-emergent adverse events, will also be provided. For the long-term extension portion of this study, exposure adjusted event rates will be summarised.

Sample size and power considerations

When designing this trial, no prior data for FMT efficacy in rheumatic patients were available. However, we found it reasonable to assume that if rheumatic patients should be willing to receive FMT as a future standardised treatment, the procedure should at least provide an effect size well beyond a moderate effect size. Consequently, we decided that at least twice as many PsA patients in the sham group should be treatment failures compared to the FMT group if the procedure should be considered clinical relevant. For a comparison of two independent binomial proportions using the Pearson's chi-squared statistic with a Chi-square approximation (a two-sided significance level of 0.05), a sample size of 40 PsA patients per group has a power of 90% (0.895) if we assume that the proportions of treatment failures are 35% (FMT-active group) and 70% (FMT-sham group), respectively. Consequently, the inclusion of 80 PsA patients allocated (1:1) to two treatment arms is believed to be sufficient to reveal any difference of clinical importance between treatment groups (i.e., an NNT <3 patients).

Assuming that there will be some attrition during the 6-month trial period, we also estimated how much drop-out would be possible while still having a reasonable statistical power (80%): a total sample size of 62 PsA patients assuming a comparable level of withdrawals (31 patients completing in each group) achieves a power of at least 0.8 with the proportion of treatment failures indicated above; i.e., even if we experience a drop-out rate of 20%, our trial will have 80% chance of detecting the intentional difference between groups.

Beyond the primary endpoint, a total sample size of 80 (with a balanced design) corresponds to a sufficient statistical power (82%) to detect a standardised mean difference of 0.65 SD units (i.e. Cohen's effect size) in any of the Patient-Reported Outcome Measures.

Randomisation, allocation concealment and blinding

The randomisation has been conducted using central-computer randomisation. Patients are randomly allocated (1:1) to receive either a FMT or a placebo saline transplant (sham procedure). The randomisation lists were generated by the trial statistician and uploaded to the REDCap database by an independent data manager who is not involved in any other aspects of the trial. Eligible patients will - after signing informed consent - be assigned randomly in permuted blocks with varying sizes of 4 and 6, according to computer-generated random numbers (SAS programming via SAS PROC PLAN), to undergo either FMT or saline (sham) procedure using stratification for centre. The randomisation of each patient will be implemented by the local trial coordinator and allocation will be concealed as this is done independent of the pre-determined sequence generation (i.e. randomisation). The patients, care providers and outcome assessors will remain unaware of the group assignments, and only de-identified codes will be used to link participants to their data during the study to maintain their confidentiality. In case of exceptional circumstances when knowledge of the treatment allocation is essential for further management of the patient, the trial secretary will reveal the assigned intervention to the treating doctor.

However, patients, trial care providers and outcome assessors will remain blinded as far as possible. Cases of unblinding will be registered and reported.

Data collection, management and confidentiality

Data will be entered via a secure web-based electronic clinical report form (eCRF) into a central REDCap[93] database hosted by Odense Patient data Explorative Network (OPEN) at Odense University Hospital. Data obtained during the clinical examination will be entered directly into the database. Also, patient questionnaires will be fulfilled directly into the database. Access to the study data will be restricted, and a password system will be utilized to control access. All information about the patients' health and other private matters is covered by confidentiality. The authorisation from the Danish Data Protection Agency has been secured.

Statistical methods

The full analysis set will consist of all randomised participants (i.e. the intention to treat [ITT] population): Participants will be analysed according to their randomised treatment group; i.e. the ITT has the consequence that participants allocated to a treatment group will be followed up, assessed and analysed as members of that group irrespective of their compliance to the planned treatment. The safety analysis set will include all patients who were randomly assigned to a study group and had exposure to a transplant (independent of group). Descriptive statistics will be provided for demographics and baseline characteristics. The summary statistics of continuous variables will include: N, mean, standard deviation, median, interquartiles, and range. All summaries presenting frequencies and incidences will include counts, percentages, and the total number of participants in the corresponding arm.

The pre-specified efficacy analyses will be based on data from the full-analysis set, which include all patients who underwent randomisation, have had their baseline measurement performed, and who have received the initial transplant (independent of group). Although proper random assignment prevents selection bias, it does not guarantee that the groups will be equivalent at baseline. Any differences in baseline characteristics are, however, the result of chance rather than bias;[94] thus, the study groups will be evaluated (and presented) at baseline for important demographic and clinical characteristics so that readers can assess how similar they are. However, only cohort studies can be subject to selection bias and confounding due to differences in baseline characteristics between the intervention and comparison groups.[95]

Our strategy for ITT analysis with incomplete observations will be based on the recommendations from White et al[96]:

1: Attempt to follow up all randomised participants, even if they withdraw from allocated treatment.

2: Perform a main analysis of all observed data (data as observed).

3: Perform sensitivity analyses to explore the effect of departures from the assumption made in the main analysis (Baseline Observation Carried Forward [BOCF] imputations, repeated measures mixed models, and multiple imputations).

This results in the following steps: Missing values will be imputed with the use of a non-responder imputation by use of the BOCF method for measurements made after baseline. Thus, missing data for dichotomous endpoints will also be imputed using a conservative "null responder" imputation, assuming the patient did not have any benefit from being enrolled in the trial (e.g., for the primary endpoint we will assume that the patient had a treatment failure which is valid based on clinical judgement even if data is not missing at random [NMAR]). Other sensitivity analyses will be including "worst" and "best" case imputation, repeated-measures and multiple-imputation analyses, using model-based approaches; repeated measures linear mixed models will also be used to model the potential group-dependent trajectories over time (i.e. Repeated Mixed Models and Multiple Imputation are valid if data is assumed Missing at Random [MAR]).

Categorical data for dichotomous end points will be analysed with the use of logistic regression with the model including treatment and centre as class effects. For continuous outcome measures analysis of covariance (ANCOVA) models will be used to analyse mean changes in continuous end points. All models will include treatment, centre, with the baseline value of the relevant variable as covariates.

Additionally, completer analyses will be performed on those who complete 6 months of treatment. During follow-up, any medical treatments which could potentially modify the intestinal microbiota including antibiotics will be reported, but will not affect the statistical analysis. Statistical estimates will be calculated as odds ratios (OR) for the dichotomous variables and difference between means for continuous outcomes reported with 95% confidence intervals (95% CI). Two-sided 95%CIs and P-values for primary, secondary and exploratory outcomes will be computed and will not be adjusted for multiplicity, but will be interpreted cautiously as this is an exploratory trial per se.

Pre-specified exploratory analyses: Stratified analyses will investigate whether the treatment effect varies with I) the faecal microbiota analyses performed at follow-up compared with baseline (+/- long-term changes in the intestinal microbiota and intestinal inflammation); and II) the demographic match (sex, age) between the stool donor and the recipient. Non-responders will represent the outcome group not fulfilling the primary outcome measure. Differences in demographics and baseline disease activity between this treatment-failure subpopulation and the remaining group will be examined to identify potential prognostic factors for poor responders. Patients not participating in the follow-up examination will be classified as "drop-outs", and if possible, the reason for not participating will be registered.

The faecal metagenomics bioinformatics analyses will be performed using comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics species,[71,72] taxonomic annotation, and extensive functional analyses based on metagenomic species which provides a superior dataset compared to the conventional analyses based on the total gene pool.[73] To identify possible associations, metagenome analysis will be correlated to all clinical parameter. We will use an L1 restricted LASSO procedure to determine the optimal number of features to be tested as described. Analysis of correlations between microbiota taxonomic or functional features, community diversity indices and sample metadata variables will be performed using Spearman correlation tests corrected for multiple tests using the Benjamini-

Hochberg false discovery rate control procedure. To control for confounders, we will use blocked Spearman tests as implemented in COIN.[97,98]

Data will be analysed with the STATA statistical package (version 15; StataCorp LP), and SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA).

Activity/assessment	Pre-study screening	Visit 1 Baseline	Week 1, 2 and 3	Visit 2 1 month	Visit 3 3 months	Visit 4 6 months
Patients	n = ?	n = 80	n = all	n = all	n = all	n = all
Screening log	x					
Inclusion/exclusion form	x					
Consent form		x				
Randomisation		x				
Study-composed questionnaire		x	x	x	x	x
Patient global (VAS 0-100 mm)		x	x	x	x	x
Patient fatigue (VAS 0-100 mm)		x	x	x	x	x
Patient pain (VAS 0-100 mm)		x	x	x	x	x
HAQ		x	x	x	x	x
BASDAI		x			x	x
BASFAI		x			x	x
DLQI		x	x	x	x	x
Gastrointestinal symptom diary		x	x	x	x	x
Eating habits questionnaire		x				
Clinical examination:						
- Height (m)		x				
- Weight (kg)		x			x	x
- Blood pressure (mmHg)		x			x	x
- Psoriasis Area Severity Index		x			x	x
- SPARCC Enthesitis Score		x			x	x
- Swollen joint count (66)		x			x	x
- Tender joint count (68)		x			x	x
- Doctors global (VAS 0-100 mm)		x			x	x
- BASMI		x			x	x
- Tender point count		x			x	x
Interview (AEs)				x	x	x
Blood sample analysis:						
- C-reactive protein (mg/L)		x		x	x	x
- Orosomucoid (g/L)		x		x	x	x
- Calprotectin		x		x	x	x
- 1,25-dihydroxyvitamin D (nmol/L)		x		x	x	x
- TSH (miu/L)		x				x
- Hgb (mmol/L)		x				x
- Triglyceride (mmol/L)		x				x
- LDL-cholesterol (mmol/L)		x				x
- HDL-cholesterol (mmol/L)		x				x
- Total-cholesterol (mmol/L)		x				x
- HbA_1C (mmol/mol)		x				x
- HLA-B27 status (+/-)		x				
- Serology tests for *Yersinia, Campylobacter*, *Salmonella* (+/-)		x				
Faecal calprotectin		x		x	x	x
Faecal microbiota analysis		x		x	x	x
Sigmoidoscopy and mucosa biopsy		x				x
Stool, blood, and urine samples (biobank)		x		x	x	x
Intestinal permeability test		x				x
Intervention (+/- FMT)		x				
Serious adverse event forms				x		

Table 1. Protocol schedule of forms and procedures

ETHICS AND DISSEMINATION

This study is designed as a proof-of-concept clinical trial and will be performed in agreement with GCP-standards, and in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration (64th, 2013). The relevance of the study, the design and the recruitment strategy were evaluated with three patient research partners (PRPs), and alterations especially in primary outcome and recruitment strategy were embedded. Furthermore, a minimum of two PRPs (participating in the study) will be involved in the discussion regarding the progress of the recruitment phase and results, and will be offered the opportunity to comment on the manuscript draft. The Regional Committees on Health Research Ethics for Southern Denmark (DK-S-20150080) and the Danish Data Protection Agency (15/41684) have approved the study protocol. The trial has been registered with ClinicalTrials.gov (NCT03058900) and important protocol modifications will be updated here. The Danish Health and Medicines Authority does not classify the FMT procedure as a medical intervention, and has had no objection to the use of FMT for this study and patient category. Thus, no GCP auditing is legally required. A report describing any potential side effects and adverse events will be submitted to the Ethics Committee yearly. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the Ethics Committee within 7 days. Based on these reports, the Ethics committee can determine to terminate the trial early. The Danish Patient Compensation Association provides compensations for patients injured in connection to medical clinical trials.

Although the Danish Health Authorities, for the time being, do not classify donor faecal microbiota as tissue, all steps of the stool donor recruitment, stool donation and FMT preparation will be in accordance with the Danish Tissue Law to ensure that the quality and safety standards laid down in the Danish Legislation BEK nr 764 of May 26, 2015 (implementing Directive 2004/23/EC) are met. Four stool donors will be recruited from the South Danish Transfusion Service & Tissue Centre, Department of Clinical Immunology, Odense University Hospital, and they will be carefully screened for potentially transmissible infections and other conditions associated with gut microbiota function before their stool can be released for FMT. Being a stool donor is voluntary, and no compensation fee will be given. Furthermore, to ensure donor traceability, each patient in the active treatment arm will only receive microbiota from one donor. Also, frozen samples will be clearly labelled with a unique donation code based on the ISBT 128 coding and labelling system, and the release of the final product will adhere to the standards for tissue and blood donation.

Due to the well-documented risk of permanent joint destruction and occurrence of extra-articular manifestations in the PsA disease course, identification of new treatment modalities and biomarkers is essential to help the physician to slow down the disease development or ultimately to prevent it. All PsA patients participating in this study have significant activity in their joint disease despite treatment with the current guideline treatment and first-line drug, MTX, for this condition. This patient population will therefore benefit greatly from new treatment options. Consequently, when weighing the pros and cons, this trial should be performed from a scientific and ethical perspective.

Dissemination will occur through presentations at national and international conferences and publications in international peer-reviewed journal(s).

DISCUSSION

Recent years have seen growing recognition of the complexity of the role of the microbiota in shaping the immune system and its potential effects for health and disease.[22,99,100] In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.[101-104] Intriguingly, an abnormal intestinal bacteria composition has been observed in PsA patients, and this association has fostered theories linking intestinal dysbiosis and PsA joint inflammation.[105] Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic diseases are causal related,[55] and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.[54,106] We expect that this double-blind, randomised, placebo-controlled trial will shed new light on this highly relevant topic.

This is the first time that the efficacy and safety of FMT is being investigated in MTX immunosuppressed patients with rheumatic diseases. Subsequently, no data on the feasibility of conducting FMTs in the rheumatological setting is, so far, available. Nor do we know whether one FMT will be sufficient, or whether it should be repeated shortly after the first intervention to normalise the alterations of the intestinal microbiota, which would expectedly enhance any potential anti-inflammatory effects. In the present proof-of-concept clinical trial, the FMT procedure is considered an add-on to the current guideline intervention and first-line drug, MTX. Therefore, from a pragmatic and ethical perspective, we have decided to perform only one FMT (or sham procedure) in each patient even if we are well aware that this approach may not be adequate to achieve long-lasting effects. Indeed, in patients with inflammatory bowel diseases it appears that performing a frequent dosing-regime repeating the FMT procedure up to five times a week for eight weeks provided the best results.[51,107,108] Hence, in contrast to the treatment of *C. difficile* infections where the microbiota is pushed past the point of homeostasis and can be restored following only one FMT,[47] the chronic nature of PsA and other autoimmune and inflammatory diseases, and the somewhat lesser degree of intestinal dysbiosis, may make the host microbiota more resistant to long-lasting modifications. Nevertheless, we hope that the FMT procedure in the present study will be sufficient to boost the effects of MTX so that the participants who are all MTX-non-responders prior to study enrolment will achieve disease control without needing to add or switch to other non-MTX medication.

In the present trial, the primary outcome measure is defined as the occurrence of treatment failure according to shared decision making between patient and physician evaluated at 6 months following the randomised intervention (FMT versus sham procedure). Shared decision making is a process in which both patient and health professional make a decision, taking into account the best evidence of available treatment options and the patient's values and preferences. This approach is considered a key element in the management of rheumatic diseases.[109] As both patients and the treating rheumatologists are blinded to the randomised intervention, the shared decision making will be unaffected by the type of transplant suspension (active or placebo) installed at baseline. Nevertheless, we acknowledge that our assumption that twice as many PsA patients in the sham group will be treatment failures is ambitious, and that we might miss a smaller and less clinically significant treatment effect of the FMT-procedure. In this

case, we hope that our secondary outcome measures will be able to detect potential trends of positive effects in PsA subdomains such as enthesitis score, dactylitis count, and PASI skin score. In addition to the primary endpoint evaluation at 6 months, patients will be asked to fill out a weekly questionnaire regarding side effects as well as skin and arthritis symptoms during the first month following the randomised intervention to reveal any short-term effects on patient-reported outcomes.

Next, only patients with active peripheral PsA will be included. One reason for this is that this will be the first time that FMT is performed on rheumatic patients. Therefore, it seems reasonable only to enrol patients who have had inadequate effect from the initial guideline treatment (MTX), and consequently, on an individual basis could benefit the most from participating in new experimental clinical trials. Also, since patients need to have at least three swollen joints, we expect that we will be able to detect treatment effects of clinical importance. The fact that we do not include recent onset treatment naive patients will, of course, limit our ability to generalise our findings unto the entire PsA patient population. Indeed, in a recent randomised controlled trial of FMT in ulcerative colitis patients, participants with a recent diagnosis (< 1 year) were statistically significantly more likely to respond to FMT compared with those with longer disease duration.[107] That patients will have to subcutaneously administer MTX for at least three months prior to study enrolment will ensure that low intestinal MTX absorption is excluded as a potential effect modifier for the poor MTX response. In addition, as many drugs, including MTX, seem to affect the intestinal microbiological millieu,[110-113] bypassing the intestine during MTX administration will ensure that no local non-disease related effects on the intestinal microbiota will occur.

A great challenge when conducting a trial of FMT is that for the present being there is a lack of both national and international recommendations guiding the regulation and the best clinical practices for donor screening, stool sample handling and preparation of the FMT suspension.[114-116] Indeed, the variability in faecal bacterial communities can complicate or undermine treatment efficacy. This variability stems from both biological variation and variation introduced by sample handling. A recent study reported that oxygen exposure degraded faecal bacterial communities, whereas freeze-thaw cycles and lag time between donor defecation and transplant preparation had much more limited effects.[117] Given that many intestinal bacteria are obligate anaerobe, including many beneficial bacteria potentially possessing anti-inflammatory effects, exposure to oxygen during the preparation of FMT may potentially compromise the therapeutic value of FMT in PsA and other inflammatory diseases. Therefore, although frozen faecal preparations of stool suspended into physiological saline and glycerol have proven just as effective as fresh stool in treating *C. difficile* infections,[118] the optimal transplant preparation method in treating inflammatory diseases remains to be established.

Our stool handling setup is in line with the prevailing practice, which includes mixing and filtration of the stool suspension and adding saline and glycerol as a cryopreservative before storage at -80 ºC.[116] In addition, we have sought to limit the oxygen exposure during transport by placing the donor stool within a plastic bag, which is subsequently put into a tightly closed small plastic container. Supplementary, during preparation the solution will not be homogenized for more than 10 seconds. Nevertheless, the lack of optimal anaerobe conditions during stool

handling could possibly undermine the therapeutic potential of our FMT procedure. Furthermore, although we aim to use 50 g of faeces for each transplant, we acknowledge that the exact weight between donations could vary with an estimated +/- 5 g. Also, due to the wide variability in microbial content in stool between donations, the content cannot be fully standardized, and may likely differ between each FMT procedure. However, to meet this challenge we will collect and store samples from each donation which will enable us to determine the microbiota composition of each donation in case some donations prove more effective than others.

Stool donor selection is another critical issue that needs to be addressed. The composition of the normal microbiota composition has only recently been mapped,[119] and the existence of a limited number of well-balanced host–microbial symbiotic states, where one or more bacteria species are considered the main functional driver(s), have been identified using clustering of metagenomic sequences.[120] Still, the most favourable donor microbiota composition for treating inflammatory diseases has yet to be determined. Therefore, it also remains to be established whether donors with a high stool bacteria diversity should be preferred over isolation of specific bacteria, or if pooled stool samples from several donors outperforms a single-donor transplant.[51,121] We have chosen to use only single donations from four different anonymous stool donors to ensure donor traceability and to enable us to identify any individual donor-specific microbial effects. Also, since host intrinsic-, environmental-, and dietary factors as well as pharmaceutical drugs have been associated with gut bacteria composition and functionality,[110,111,122,123] the donors must eat a balanced diet, not be overweight or take any medications or be physical or psychological stressed, smoke or consume alcohol during the donation period to limit the risk of transferring "abnormal" microbiota to the recipients. These donor criteria have been set for safety reasons, and we acknowledge, that this could potentially limit the inter-donor microbiota diversity due to shared lifestyle characteristics.

Another factor to keep in mind is the concept of matching donor and recipient, which may be of importance for enhancing the colonisation capabilities of the donor microbial communities. In fact, Rossen et al[108] did find that in patients with ulcerative colitis, the microbiota of FMT responders shifted to their respective donors, whereas non-responders did not. Li et al[124] reported that donor bacteria strains established extensively in the recipient and persisted for at least 3 months with a negligible decline of donor-strain populations detected between 45 days and 3 months following FMT in metabolic syndrome patients. However, they also found that recipients receiving the same donor transplant displayed varying degrees of microbiota transfer, indicating individual patterns of microbiome resistance and donor-recipient compatibilities. In addition, host genetics is known to effect the gut microbiota,[125] and animal models have shown that sex[126] and age[127] also can be potentially modifiers of the gut bacteria composition. These observations may prove to be of importance for the outcome of FMT in inflammatory diseases.[128] However, whether sex- and/or age-matching between donor and recipient is crucial for a successful FMT in humans remains to be enlighten. Therefore, in the present study, no donor-recipient matching will be conducted. However, a subgroup analysis will be performed to reveal any trend that could indicate better results in sex- or age-match cases.

Furthermore, as the interactions between the microbiota and the host are influenced by cooperation and competition between pathogenic and commensal microbes and multiple

environmental variables, the lifestyle of the recipient following the FMT may be of importance. Nevertheless, due to the uncertainty of how to define the optimal lifestyle and the lack of knowledge on how different lifestyle factors may interfere with the microbiota, we have decided that the patients in the present study will not have to adhere to any predefined lifestyle "regime" or diet following the randomised intervention. However, every participant will fulfil an eating habit questionnaire at the beginning of the trial.

Finally, non-bacteria microorganisms such as bacteriophages, viruses and fungi may also be of importance when targeting components of the microbiota or host cells for therapeutic purposes.[129-131] Other complicating factors may include the composition of other microbiological niches such as the oral, lung, genitourinary, and skin microbiota.[132,133] Indeed, the latter could likely prove to be of significance in patients with skin psoriasis. However, these factors will not be assessed in the present study.

In conclusion, this trial has the potential to substantially expand the growing body of literature on the role of the intestinal microbiota in general and PsA in particular. Thereby we anticipate that this study will enhance our understanding of cause and effect. The results of this study, when completed, may be exploited for biomarker discovery, and for diagnostic and therapeutic purposes.

AUTHORS' CONTRIBUTION

T. Ellingsen, M.S. Kragsnaes, R. Christensen, and J. Kjeldsen conceived and developed the idea for the study. T. Ellingsen and M.S. Kragsnaes are the principal investigators and wrote the first study protocol draft. T. Ellingsen and M.S. Kragsnaes were responsible for all communication with the scientific ethical committee, the Danish Data Protection Agency and the National Health Board. T Ellingsen is the responsible party and sponsor. M.S. Kragsnaes, T. Ellingsen, H.C. Horn, J.K. Pedersen, H.L. Munk, and U. Fredberg sat up the inclusion and exclusion criteria for the psoriatic arthritis patients, and the latter five rheumatologists are conducting the clinical examinations. J. Kjeldsen, F.M. Pedersen, and H. Glerup discussed and planned the FMT procedure, and they are conducting the FMTs and the sigmoidoscopies (obtaining mucosae tissue samples). D.K. Holm and H.M. Holt helped set up the donor screening programme, and they were responsible for conducting this programme and performing the microbiological and immunological tests. V. Andersen and K. Kristiansen are responsible for the omics and microbiome analyses, and have advised on how the tissue collection should be performed and what kind of tissue would be relevant to collect. R. Christensen has written the statistical analysis plan and will be responsible for the final statistical analyses. In conclusion, all participants designated as authors have contributed to the conception and design of the study, and they have critically either drafted or revised the first draft of the study protocol and the protocol paper. Also, all authors have approved the final version before submission.

REGISTRATION

The trial has been registered with ClinicalTrials.gov (NCT03058900).

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COMPETING INTEREST STATEMENT

None of the team members of this research project has declared any potential conflict of interest.

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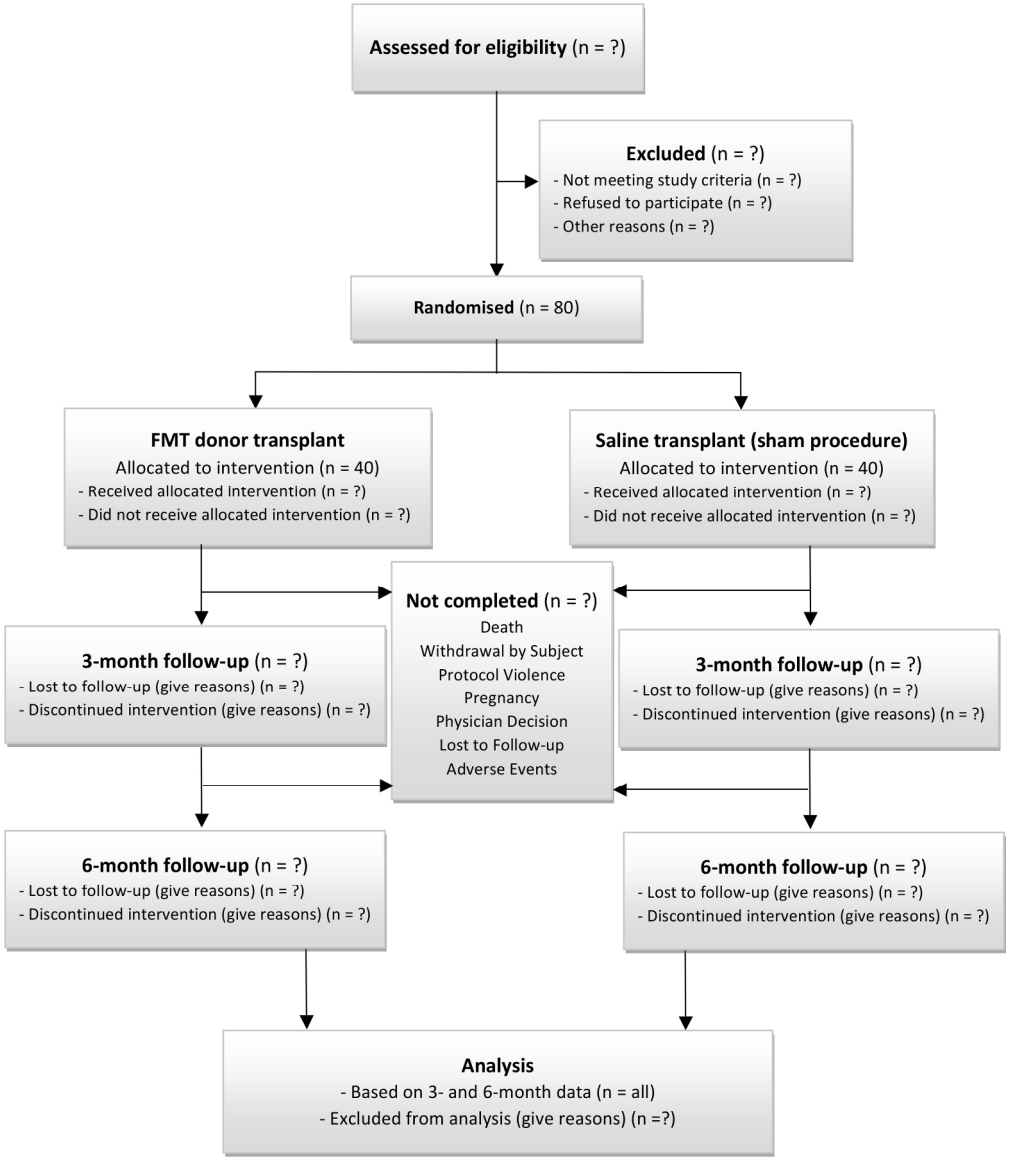

Figure 1. Flow diagram of the randomised, placebo-controlled trial.

198x236mm (300 x 300 DPI)

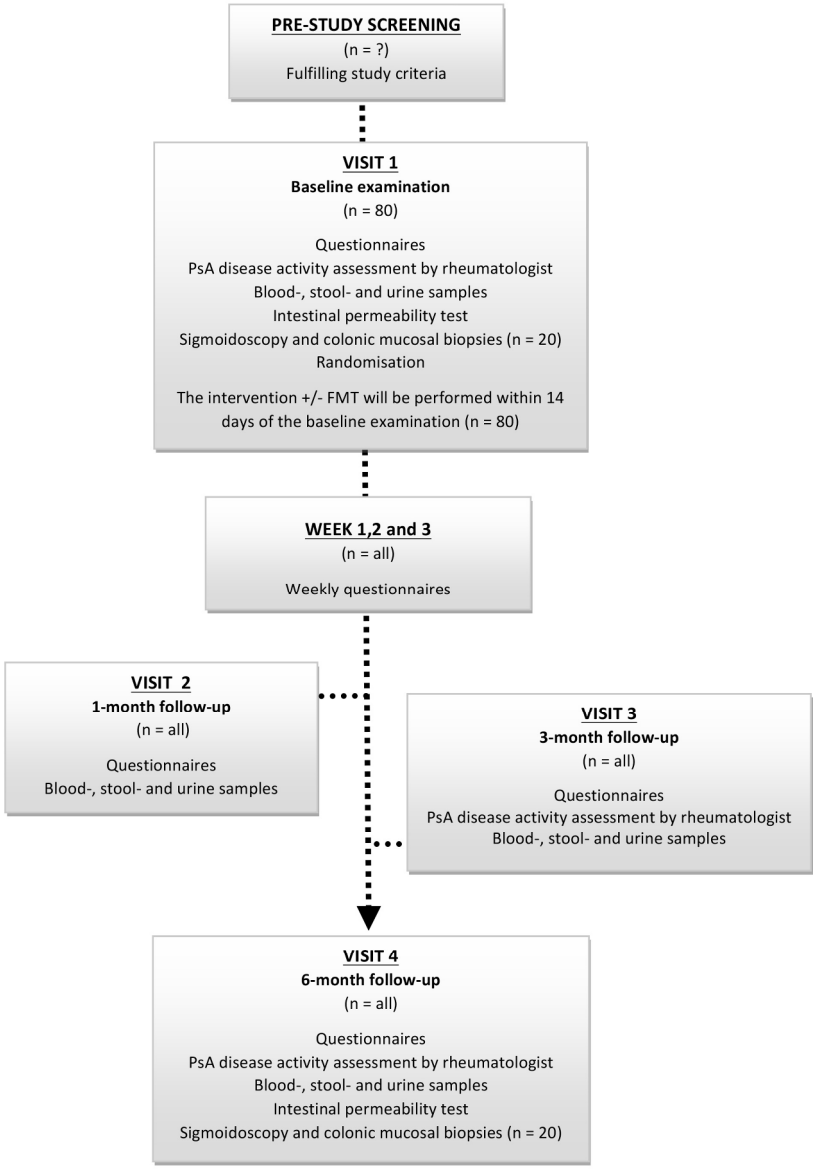

Figure 2. Participation timeline and characteristics of each visit.

160x237mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	1-23
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 and 22
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	22

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4-5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Not applicable
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8 and 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13-14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	14
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	14
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	14
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	18
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	18
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	18

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	14
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Not applicable
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	18
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	22
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	18
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10-11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised, placebo-controlled trial

The FLORA trial

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Efficacy and safety of faecal microbiota transplantation in patients with psoriatic arthritis: protocol for a 6-month, double-blind, randomised, placebo-controlled trial

The FLORA trial

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ABSTRACT

Introduction: An unbalanced intestinal microbiota may mediate activation of the inflammatory pathways seen in psoriatic arthritis (PsA). A randomised, placebo-controlled trial of faecal microbiota transplantation (FMT) infused into the small intestine of PsA patients with active peripheral disease who are non-responsive to methotrexate (MTX) treatment will be conducted. The objective is to explore clinical aspects associated with FMT performed in PsA patients.

Methods and analysis: The FLORA trial is a randomised, two-centre stratified, double-blind (patient, care provider and outcome assessor), placebo-controlled, parallel-group study. Eighty patients will be included and randomised (1:1) to either placebo (saline) or FMT provided from an anonymous healthy donor. Throughout the study, both groups will continue the weekly self-administered subcutaneous (s.c.) MTX treatment, remaining on the pre-inclusion dosage (15-25 mg/week). The clinical measures of psoriasis and PsA disease activity used include the Health Assessment Questionnaire (2-page DI-HAQ), the Dermatology Quality of Life Index (DLQI), the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index, the Psoriasis Area Severity Index (PASI), a dactylitis digit count, a swollen/tender joint count (66/68), plasma C-reactive protein as well as visual analogue scales for pain, fatigue, and patient and physician global assessments. The primary endpoint is the proportion of patients who experience treatment failure during the 6-month trial period. The number of adverse events will be registered throughout the study.

Ethics and dissemination: This is a proof-of-concept clinical trial and will be performed in agreement with Good Clinical Practice (GCP) standards. Approvals have been obtained from the local Ethics Committee (DK-S-20150080) and the Danish Data Protection Agency (15/41684). The study has commenced in May 2017. Dissemination will be through presentations at national and international conferences and through publications in international peer-reviewed journal(s).

Trial registration number at ClinicalTrials.gov: NCT03058900

Strengths and limitations of this study

- This is a double-blind, randomised, placebo-controlled trial.
- Subcutaneously administered MTX treatment.
- The primary endpoint is based on shared decision-making between patient and physician.
- No feasibility data regarding FMT in rheumatic patients were available when the trial was designed.
- A limitation of the study is that the content of the faecal transplant suspension cannot be fully standardised.

INTRODUCTION

Emerging data suggest a causal relationship between the intestinal microbiota and spondyloarthritis (SpA), thus, linking dysbiosis of the complex microbial communities with SpA pathogenesis.[1-5] Psoriatic arthritis (PsA) is one of five SpA categories in adults which also include ankylosing spondylitis, undifferentiated arthritis, reactive arthritis and arthritis associated with inflammatory bowel disease. While the association between the gut and the latter two disorders is well established,[6] only very recently, studies evaluating the faecal microbiota and the presence of subclinical gut inflammation in PsA patients have coupled this disease to a perturbation of the intestinal microbiota composition.[7-12]

PsA is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum and a varied disease course.[13] The clinical manifestations include peripheral arthritis, enthesitis and/or spondylitis combined with more or less severe psoriatic skin involvement, nail psoriasis, and dactylitis.[14] Nearly half of the patients with both early and established PsA also present with extra-musculoskeletal manifestations which can include bowel (16%), ocular, cardiovascular or urogenital involvement.[15] Without disease modifying intervention, 40-60% of PsA patients will develop erosive and deforming joint damage within a few years of disease onset.[16] Methotrexate (MTX) has long been the preferred conventional synthetic disease-modifying anti-rheumatic drug (csDMARD) for initial therapy.[17] However, the evidence for MTX in PsA is poor, and a substantial number of patients does not benefit from such treatment.[18] Currently, other treatment options may include biological agents such as tumour necrosis factor (TNF-α) inhibitors aiming to block some of the downstream molecular pathways driving the disease.[19] Still, these drugs do not target the cause of PsA, which is believed to be multifactorial comprising genetic, immunological and environmental factors.[20] The interplay between these complex aetiological factors has yet to be fully understood.[21,22]

The classic pathophysiological concept of PsA is that it is an autoimmune disease of the skin and joints and that the pathological processes at both sites are driven by inflammatory responses involving the innate immune system, natural killer cells, T cells, and the expression of pro-inflammatory cytokines, including TNF-α, interleukin (IL)-1, interferon-γ, IL-6, IL-12, IL-15, IL-18 and the IL-17/IL-23 axis.[23-27] However, although microbial agents including dormant bacteria, mycobacteria, bacterial products and viral antigens have been implicated as potential initiators,[28,29] the true pathophysiological factors triggering the dysregulated immunological cascade underlying the disease remain to be identified.

Intriguingly, it has recently been suggested that mucosal sites exposed to a high load of bacterial antigens, in particular the gastrointestinal tract, may represent the initial site of immunological tolerance break in PsA.[30] Indeed, under normal conditions the host and the microbiota live in harmony and benefit from their mutualistic relationship. However, alterations of the normal intestinal microbiota can affect mucosal immunity which, in turn, can induce local inflammation and elicit systemic effects at distant sites.[31] Mechanisms through which the intestinal microbiota may be involved in the pathogenesis of PsA include an abnormal activation of the gut-associated lymphoid tissue,[32] a decrease in regulatory T cell activity,[33] and/or an altered mucosal permeability thus compromising the capacity of the intestine to provide adequate containment of luminal microorganisms and molecules.[34,35] In support of these theories, several studies have documented subclinical gut inflammation in PsA patients.[36-41] Moreover, a recent

study reported that several intestinal bacteria including *Akkermansia* and *Ruminococcus* were practically absent in PsA patients. These commensal bacteria are, in fact, known to play an important role in maintaining gut homeostasis.[42]

Rationale

If the gut microbiota is the initiator and/or mediator of the common inflammatory pathways seen in PsA,[8] modifying the intestinal microbiota could be a novel treatment strategy for this disease.[1-3,43] Faecal microbiota transplantation (FMT) is currently being used to restore the balance of the intestinal flora.[44,45] Particularly, this procedure has demonstrated more than 90% clinical resolution of recurrent or refractory *Clostridium difficile* infections.[46-50] Also, multiple FMTs seem to be able to induce remission in patients with inflammatory bowel disease (IBD).[51] Due to these results, FMT is now being tested as a potential novel treatment for other gastrointestinal and extra-intestinal diseases.[52] To the best of our knowledge, no study has yet ascertained the efficacy and safety of FMT in patients with inflammatory rheumatic diseases.

Evidence-based research

To avoid waste of research no new studies should be initiated without a systematic review of the existing evidence.[53] We performed a pragmatic search in the biomedical literature via Pubmed combining different related MeSH terms: ("Microbiota"[Mesh] OR "Fecal Microbiota Transplantation"[Mesh] OR "Faecal Microbiota Transplantation"[Mesh] OR "Gastrointestinal Microbiome"[Mesh]) AND (arthritis[tiab] OR "Arthritis"[Mesh] OR "Arthritis, Psoriatic"[Mesh] OR “Arthritis, Reactive”[Mesh] OR “Spondylarthritis”[Mesh] OR "Arthritis, Gouty"[Mesh] OR "Arthritis, Rheumatoid"[Mesh] OR "Psoriasis"[Mesh]). From the search revealing 122 citations it became clear that the majority of papers were reviews/editorials (74 [61%]), where the overall conclusion was that the main challenges are to uncover the cause-effect relationship between the intestinal microbiota and rheumatic diseases, and to investigate the potential of microbiome-targeting strategies.[1,3,5,6,20,32,43,54-60] Also from the published literature it became evident that to date only nine clinical interventional studies trying to modify the intestinal microbiota in arthritis patients have been performed: One study in SpA patients (n = 63),[61] and one study in enthesis-related arthritis (n = 8) reported no beneficial effects of probiotic therapy,[62] whereas one study in juvenile idiopathic arthritis testing exclusive enteral nutrition administration (n = 7) found a moderate anti-inflammatory effect on active joints.[63] Five placebo-controlled trials of probiotic therapy in rheumatoid arthritis patients[64-68] (sample size between 26 and 60 patients) reported mixed results.[69] However, two of these studies demonstrated positive clinical effects of probiotic therapy which included improvement in HAQ-DI pain scale,[65] improvement in the Disease Activity Score of 28 joints (DAS-28), and improvement on the C-reactive protein concentrations.[66] No clinical trials performing FMT on arthritic patients were identified.

Objective

The objective of this randomised trial is to explore whether FMT is more effective than placebo in reducing disease activity in PsA patients with active peripheral arthritis concomitantly treated with weekly subcutaneously administered MTX. In addition, extensive bacteria taxonomic and

metagenomic analyses will be performed on faecal samples before and after the FMT to get an indication of the functional capacity of the intestinal microbiota.

METHODS AND ANALYSIS

Trial design

This is a randomised – patient, physician and outcome-assessor blinded, placebo-controlled, 6-month trial, which will be followed by an open-label extension period for a minimum of 2 years. Patients will be randomly assigned in a 1:1 ratio to receive FMT or placebo (sham procedure). Outcome assessment will be based on follow-up by a rheumatologist and is scheduled to occur after 3 and 6 months (with the latter being the primary end-point evaluation), see Figure 1 and Figure 2.

Participants

Recruitment will take place at Danish rheumatology outpatient clinics, and patients fulfilling the eligibility criteria will be offered participation. No treatment with biologics within 6 months, and no systemic and/or local intra-articular or peritendinous steroid injections, or non-MTX csDMARD treatment, or antibiotics are allowed within 3 months prior to inclusion. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) must be paused within 14 days of study inclusion. Patients, who do not wish to participate, will be characterised by sex and age. The recruitment has commenced in May 2017 and will continue until 2019.

Psoriatic arthritis patients

A total of 80 PsA patients will be enrolled, and they will have to meet the following eligibility criteria:

Inclusion criteria:

- Diagnosis of PsA according to the Classification Criteria for Psoriatic Arthritis (CASPAR).[70]
- Presence of active peripheral arthritis defined as ≥ 3 swollen joints.
- Subcutaneously administered MTX treatment (≥ 15mg/week (maximal tolerable dosage)) for a minimum of 3 months prior to study inclusion.
- Age 18 to 70 years.

Exclusion criteria:

- Other inflammatory rheumatic diseases than PsA.
- Current axial disease activity or severe peripheral joint activity demanding immediate change of treatment or contraindicating placebo treatment for 6 months.
- Inflammatory bowel disease, coeliac disease, food allergy, or other intestinal diseases.
- Current cancer or severe chronic infections.

- History of severe MTX toxicity or allergic reactions.
- Biological treatment within 6 months prior to inclusion.
- Non-MTX DMARD treatment within 3 months prior to inclusion.
- Systemic and/or local intra-articular or peritendinous steroid injections within 3 months prior to inclusion.
- NSAIDs within 14 days prior to inclusion.
- Antibiotics within 3 months prior to inclusion.
- Pregnant or breastfeeding women.
- Not wishing to participate or unsuited for project evaluation.

Stool donors

The stool donor corps will consist of four anonymous (to the recipient) donors who must be healthy as assessed by a screening questionnaire, and be active members of the Danish blood donor corps, age 25 to 55, body mass index between 18.5 and 25 kg/m^2, and an average alcohol intake less than 7 (women) or 14 (men) units per week. No alcohol intake within a week of donation is allowed, and no systemic medication including antibiotics and NSAIDs 6 months prior to the donation are allowed. The donor must eat a balanced diet (no extreme low- or high-calorie diets), and must not be in a stressful life period. Before joining the stool donor corps, each potential donor will go through a screening process including stool analyses for faecal calprotectin and enteric pathogens (*Aeromonas, Campylobacter, C. difficile*, diarrhoeagenic *Escherichia coli, Salmonella, Shigella, Vibrio, Yersinia enterocolitica,* and multidrug-resistant bacteria, parasites including microscopy of ova and cysts, *Entamoeba histolytica/dispar* (DNA), *Cryptosporidium* (DNA) and Giardia (DNA), sapovirus (RNA), rotavirus (RNA), human astrovirus (RNA), human adenoviruses (DNA) and noroviruses (RNA), a *Helicobacter pylori* breath test, blood tests for C-reactive protein (CRP) (acceptable level: < 6.0 mg/L), white blood cell count (acceptable range: 3.50-8.80 10^9/L), haemoglobin (acceptable range: 8.3-10.5 mmol/L), albumin (acceptable range: 36-50 g/L), alanine aminotransferase (ALAT) (acceptable range: 10-70 U/L), glomerular filtration rate (eGFR) (acceptable level: > 59 mL/min), and coeliac disease, and blood test for infectious agents including current infection with Epstein-Barr virus (IgM) and cytomegalovirus (IgM), hepatitis A, B, C and E, tuberculosis (QuantiFERON® TB-Gold test), syphilis, human immunodeficiency virus (ab HTLV1/2), *E. histolytica* (antibodies) and *Strongyloides* (antibodies), and a urine test for *Chlamydia Trachomatis* and *Neisseria gonorrhoeae* (DNA/RNA). After passing the screening tests, the donor will donate stool for the next month after which, the donor will have to pass the screening programme once more before the stool can be released for transplantation.

Interventions

Overall study interventions

The FMT will be an add-on strategy for PsA patients with active joint disease despite ongoing treatment with weekly subcutaneously administered MTX. Therefore, all enrolled PsA patients will continue their MTX treatment throughout the study, and they will remain on the same individual dosage that they received at the time of study inclusion (a minimum of 15 mg/week cf. the patient inclusion criteria) in addition to folic acid supplement. Paracetamol and tramadol in recommended dosages are allowed during the trial but no NSAIDs can be taken.

Active and sham comparator

Patients will be randomised into two groups with an allocation ratio of active-to-placebo treatment of 1:1. The active comparator group (n = 40) will have an FMT with healthy donor faeces-suspension (250 mL) containing 50 g donor faeces, saline (NaCl 0.9%) and glycerol (10%), whereas, the sham comparator group (n = 40) will be treated with an identical appearing sham procedure where the transplant solution will consist of 250 mL brown coloured (brown food colourant) isotonic saline (NaCl 0.9%).

Preparing the FMT suspension

Donors will collect faeces at home and transport it in a cooling bag to the study site within 1 hour. Faeces will be sieved to remove particulate material, followed by dilution in sterile saline (0.9% NaCl) and 10% glycerol. The FMT suspension will be stored at - 80 ^{o}C until use. On the day of the FMT transplantation, the transplant suspension (250 mL) will be thawed to 37 ^{o}C and subsequently apportioned into five 50 mL syringes.

FMT procedure

The FMT will take place within 14 days (preferably 7 days) of the baseline clinical examination. The evening prior to the FMT, patients will take one dose (40 mg) of oral proton-pump inhibitor. They will meet at the Department of Gastroenterology after a six-hour fast. A total of 250 mL transplant suspension (active or placebo) will be installed in the duodenum using an oral-duodenal tube. The correct placement of the tube will be confirmed using gastroscopic guidance.

Treatment strategy for non-responders

Patients who present with increased or unacceptable disease activity during the 6-month trial period will, depending on the clinical presentation, be offered another treatment strategy which may include local intra-articular steroid injections, change to another csDMARD or biological treatment. If the patient accepts such treatment changes, this will be characterised as FMT treatment failure according to the primary outcome definition (one intra-articular steroid injection is allowed).

MTX toxicity and drop-outs

Blood tests for MTX toxicity will be performed in accordance with our current clinical practice. In case of MTX toxicity, severe side effects, pregnancy, or occurrence of infectious disease or other diseases that contraindicate MTX treatment, MTX dosage will be decreased or the treatment will be paused. These patients will remain in the study (unless their condition contraindicates this),

and they will be analysed as members of the treatment group to which they were randomised using intention-to-treat-type analyses.

Collection of faecal samples and metagenomics analysis

Fresh faecal samples will be collected by the patient at home using an EasySampler® stool collection kit within 24 hours prior to the study visit. Samples will be stored in the patient's freezer until transport to the study site. During transport, samples will be kept on ice in a cooling bag. Upon arrival to the study site, samples will immediately be transferred to the biobank and stored at -80°C. Bacterial DNA will be extracted from the faecal samples following established standard protocols including bead beating using a NucleoSpin soil kit (Macherey-Nagel, Germany) according to manufacturer's instructions. DNA will be sequenced using the BGISEQ-500 Platform which was recently benchmarked against the Illumina platforms showing excellent intra-platform reproducibility and less GC bias than observed using the Illumina platforms.[71] The faecal metagenomics bioinformatics analyses will be performed using comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics species,[72,73] taxonomic annotation, and extensive functional analyses based on metagenomic species which provides a superior dataset compared to the conventional analyses based on the total gene pool.[74]

Intestinal permeability test

After an overnight fasting, patients will provide a urine sample before ingesting 100 mL water containing 10 g of lactulose and 5 g of D-mannitol. All the urine passed in the subsequent 5 hours will be collected into a 2 L plastic container containing 1 mL of chlorohexidine (20 mg/mL) as a preservative. After 3- and 5 hours, the volume of the urine will be measured and a small volume (10 mL) will be preserved and stored at -80°C until analysis. No food or drinking (except for water) will be allowed during the test.[75,76]

Outcomes

Primary outcome measure:

Treatment failure [Time Frame: 6 months (+/- 14 days)]

Proportion of patients in each group who experience treatment failure according to shared decision making between patient and rheumatologist defined as at least one of the following:

- Need for more than one intra-articular glucocorticoid injection due to disease activity.
- Need for change to other csDMARDs (e.g., oral leflunomide or sulfasalazin) according to the updated Danish treatment guideline due to disease activity.
- Need for biologic treatment according to the updated Danish treatment guideline due to severe disease activity.

Secondary outcome measures:

Change from baseline in the Short Health Assessment Questionnaire (2-page HAQ)[77,78]

[Time Frame: Baseline, 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Dermatology Life Quality Index (DLQI) Questionnaire[79] [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Changes from baseline in patient reported gastrointestinal side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Other non-gastrointestinal patient reported side effects [Time Frame: 1 week, 2 weeks, 3 weeks, 4 weeks, 3 months (+/- 7 days), 6 months (+/- 14 days)]

Proportion of patients in each group achieving the American College of Rheumatology (ACR)[80] Response Criteria [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

I. ACR20 response criteria[81]

II. ACR50 response criteria[82]

III. ACR70 response criteria[82]

Proportion of patients in each group achieving the Psoriatic Arthritis Response Criteria (PsARC)[80] [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index[68] in the subset of patients who have enthesitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the Psoriasis Area Severity Index (PASI)[83] in the subset of patients who have skin psoriasis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Change from baseline in the number of digits affected with dactylitis in the subset of patients who have dactylitis at baseline [Time Frame: 3 months (+/- 7 days), 6 months (+/- 14 days)]

Number of adverse events in each group [Time Frame: 6 months (+/- 14 days)]

Number of adverse events in each group leading to discontinuation [Time Frame: 6 months (+/- 14 days)]

Number of patients with at least one adverse event in each group [Time Frame: 6 months (+/- 14 days)]

Tertiary (exploratory secondary) outcomes: Proportion of patients in each group achieving changes in plasma CRP, changes in tender point count,[84] changes in faecal bacteria composition and metabolism, changes in intestinal permeability, changes in plasma orosomucoid, changes in plasma and faecal calprotectin,[85] changes in serum 1,25-dihydroxyvitamin D, changes in cardiovascular risk factors including Body Mass Index (BMI), blood pressure, plasma triglyceride, plasma LDL-cholesterol, plasma HDL-cholesterol, plasma total-cholesterol, and HbA_1C levels, changes in specific circulating inflammatory markers (i.e. cytokines, adipokines, and chemokines), and macroscopic and microscopic inflammatory changes of the colonic mucosa, see Table 1.

Safety

The most frequently reported adverse events (AEs) related to FMT are vomiting, belching, mild diarrhoea, abdominal cramping, transient fever and elevated C-reactive protein on the day of the procedure.[86] A recent systematic review on the adverse events of FMT identified 50 relevant studies with a total of 1,089 patients. In this review, the incidences of serious adverse events (SAEs) for FMT were 2.0% and 6.1% for upper and lower gastrointestinal routes, respectively. The SAEs that probably or possibly were related to FMT included infections (0.7%), IBD flare (0.6%), death (0.3%), auto-immune diseases and FMT procedure related injury.[87] Although most of the patients included in this review suffered from severe gastrointestinal diseases (*C. difficile* infection and/or IBD), these findings warrant caution when performing FMT; especially when introducing the procedure in a new patient population. In addition, the potential long term side effects following FMT remains largely unknown.[88] Still, when strict donor screening is conducted and the procedure is performed by experienced practitioners, FMT is in general considered safe, and even elderly patients with a poor medical condition and multiple comorbidities as well as immunosuppressed patients have been proven to tolerate the FMT procedure well.[89-93]

In the present study, we will carefully monitor and evaluate safety by means of open assessment of AEs. All reported or observed AEs are recorded by the investigators, and will be monitored until resolution, stabilisation or until it has been shown that the study intervention is not the cause. The National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 (NIH publication # 09-7473), will be used to grade the severity of adverse events. Gastrointestinal side effects (nausea, vomiting, abdominal pain, number of stools pr. week, stool type (Bristol Stool Chart), blood or mucus in the stool) will be registered by the patients once a week for the first month following the randomised intervention. Routine blood screening for MTX toxicity will normally be performed at week 4, 10, 16, 22 but can be more frequent if decided by the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Subject incidence rates of all treatment-emergent AE will be tabulated by system organ class and preferred term. Tables of fatal AEs, SAEs, AEs leading to withdrawal from study, and significant treatment-emergent adverse events, will also be provided. For the long-term extension portion of this study, exposure adjusted event rates will be summarised.

Sample size and power considerations

When designing this trial, no prior data for FMT efficacy in rheumatic patients were available. However, we found it reasonable to assume that if rheumatic patients should be willing to receive FMT as a future standardised treatment, the procedure should at least provide an effect size well beyond a moderate effect size. Consequently, we decided that at least twice as many PsA patients in the sham group should be treatment failures compared to the FMT group if the procedure should be considered clinical relevant. For a comparison of two independent binomial proportions using the Pearson's chi-squared statistic with a Chi-square approximation (a two-sided significance level of 0.05), a sample size of 40 PsA patients per group has a power of 90% (0.895) if we assume that the proportions of treatment failures are 35% (FMT-active group) and 70% (FMT-sham group), respectively. Consequently, the inclusion of 80 PsA patients allocated (1:1) to two treatment arms is believed to be sufficient to reveal any difference of clinical importance between treatment groups (i.e., an NNT <3 patients).

Assuming that there will be some attrition during the 6-month trial period, we also estimated how much drop-out would be possible while still having a reasonable statistical power (80%): a total sample size of 62 PsA patients assuming a comparable level of withdrawals (31 patients completing in each group) achieves a power of at least 0.8 with the proportion of treatment failures indicated above; i.e., even if we experience a drop-out rate of 20%, our trial will have 80% chance of detecting the intentional difference between groups.

Beyond the primary endpoint, a total sample size of 80 (with a balanced design) corresponds to a sufficient statistical power (82%) to detect a standardised mean difference of 0.65 SD units (i.e. Cohen's effect size) in any of the Patient-Reported Outcome Measures.

Randomisation, allocation concealment and blinding

The randomisation has been conducted using central-computer randomisation. Patients are randomly allocated (1:1) to receive either a FMT or a placebo saline transplant (sham procedure). The randomisation lists were generated by the trial statistician and uploaded to the REDCap database by an independent data manager who is not involved in any other aspects of the trial. Eligible patients will - after signing informed consent - be assigned randomly in permuted blocks with varying sizes of 4 and 6, according to computer-generated random numbers (SAS programming via SAS PROC PLAN), to undergo either FMT or saline (sham) procedure using stratification for centre. The randomisation of each patient will be implemented by the local trial coordinator and allocation will be concealed as this is done independent of the pre-determined sequence generation (i.e. randomisation). The patients, care providers and outcome assessors will remain unaware of the group assignments, and only de-identified codes will be used to link participants to their data during the study to maintain their confidentiality. In case of exceptional circumstances when knowledge of the treatment allocation is essential for further management of the patient, the trial secretary will reveal the assigned intervention to the treating doctor. However, patients, trial care providers and outcome assessors will remain blinded as far as possible. Cases of unblinding will be registered and reported.

Data collection, management and confidentiality

Data will be entered via a secure web-based electronic clinical report form (eCRF) into a central REDCap[94] database hosted by Odense Patient data Explorative Network (OPEN) at Odense University Hospital. Data obtained during the clinical examination will be entered directly into the database. Also, patient questionnaires will be fulfilled directly into the database. Access to the study data will be restricted, and a password system will be utilized to control access. All information about the patients' health and other private matters is covered by confidentiality. The authorisation from the Danish Data Protection Agency has been secured.

Statistical methods

The full analysis set will consist of all randomised participants (i.e. the intention to treat [ITT] population): Participants will be analysed according to their randomised treatment group; i.e. the ITT has the consequence that participants allocated to a treatment group will be followed up, assessed and analysed as members of that group irrespective of their compliance to the planned treatment. The safety analysis set will include all patients who were randomly assigned to a study group and had exposure to a transplant (independent of group). Descriptive statistics will be provided for demographics and baseline characteristics. The summary statistics of continuous variables will include: N, mean, standard deviation, median, interquartiles, and range. All summaries presenting frequencies and incidences will include counts, percentages, and the total number of participants in the corresponding arm.

The pre-specified efficacy analyses will be based on data from the full-analysis set, which include all patients who underwent randomisation, have had their baseline measurement performed, and who have received the initial transplant (independent of group). Although proper random assignment prevents selection bias, it does not guarantee that the groups will be equivalent at baseline. Any differences in baseline characteristics are, however, the result of chance rather than bias;[95] thus, the study groups will be evaluated (and presented) at baseline for important demographic and clinical characteristics so that readers can assess how similar they are. However, only cohort studies can be subject to selection bias and confounding due to differences in baseline characteristics between the intervention and comparison groups.[96]

Our strategy for ITT analysis with incomplete observations will be based on the recommendations from White et al[97]:

1: Attempt to follow up all randomised participants, even if they withdraw from allocated treatment.

2: Perform a main analysis of all observed data (data as observed).

3: Perform sensitivity analyses to explore the effect of departures from the assumption made in the main analysis (Baseline Observation Carried Forward [BOCF] imputations, repeated measures mixed models, and multiple imputations).

This results in the following steps: Missing values will be imputed with the use of a non-responder imputation by use of the BOCF method for measurements made after baseline. Thus, missing data for dichotomous endpoints will also be imputed using a conservative "null responder" imputation, assuming the patient did not have any benefit from being enrolled in the

trial (e.g., for the primary endpoint we will assume that the patient had a treatment failure which is valid based on clinical judgement even if data is not missing at random [NMAR]). Other sensitivity analyses will be including “worst” and “best” case imputation, repeated-measures and multiple-imputation analyses, using model-based approaches; repeated measures linear mixed models will also be used to model the potential group-dependent trajectories over time (i.e. Repeated Mixed Models and Multiple Imputation are valid if data is assumed Missing at Random [MAR]).

Categorical data for dichotomous end points will be analysed with the use of logistic regression with the model including treatment and centre as class effects. For continuous outcome measures analysis of covariance (ANCOVA) models will be used to analyse mean changes in continuous end points. All models will include treatment, centre, with the baseline value of the relevant variable as covariates.

Additionally, completer analyses will be performed on those who complete 6 months of treatment. During follow-up, any medical treatments which could potentially modify the intestinal microbiota including antibiotics will be reported, but will not affect the statistical analysis. Statistical estimates will be calculated as odds ratios (OR) for the dichotomous variables and difference between means for continuous outcomes reported with 95% confidence intervals (95% CI). Two-sided 95%CIs and P-values for primary, secondary and exploratory outcomes will be computed and will not be adjusted for multiplicity, but will be interpreted cautiously as this is an exploratory trial per se.

Pre-specified exploratory analyses: Stratified analyses will investigate whether the treatment effect varies with I) the faecal microbiota analyses performed at follow-up compared with baseline (+/- long-term changes in the intestinal microbiota and intestinal inflammation); and II) the demographic match (sex, age) between the stool donor and the recipient. Non-responders will represent the outcome group not fulfilling the primary outcome measure. Differences in demographics and baseline disease activity between this treatment-failure subpopulation and the remaining group will be examined to identify potential prognostic factors for poor responders. Patients not participating in the follow-up examination will be classified as "drop-outs", and if possible, the reason for not participating will be registered.

The faecal metagenomics bioinformatics analyses will be performed using comprehensive pipelines including the assembly of metagenomics linkage groups/metagenomics species,[72,73] taxonomic annotation, and extensive functional analyses based on metagenomic species which provides a superior dataset compared to the conventional analyses based on the total gene pool.[74] To identify possible associations, metagenome analysis will be correlated to all clinical parameter. We will use an L1 restricted LASSO procedure to determine the optimal number of features to be tested as described. Analysis of correlations between microbiota taxonomic or functional features, community diversity indices and sample metadata variables will be performed using Spearman correlation tests corrected for multiple tests using the Benjamini-Hochberg false discovery rate control procedure. To control for confounders, we will use blocked Spearman tests as implemented in COIN.[98,99]

Data will be analysed with the STATA statistical package (version 15; StataCorp LP), and SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA).

Activity/assessment	Pre-study screening	Visit 1 Baseline	Week 1, 2 and 3	Visit 2 1 month	Visit 3 3 months	Visit 4 6 months
Patients	n = ?	n = 80	n = all	n = all	n = all	n = all
Screening log	x					
Inclusion/exclusion form	x					
Consent form		x				
Randomisation		x				
Study-composed questionnaire		x	x	x	x	x
Patient global (VAS 0-100 mm)		x	x	x	x	x
Patient fatigue (VAS 0-100 mm)		x	x	x	x	x
Patient pain (VAS 0-100 mm)		x	x	x	x	x
HAQ		x	x	x	x	x
BASDAI		x			x	x
BASFAI		x			x	x
DLQI		x	x	x	x	x
Gastrointestinal symptom diary		x	x	x	x	x
Eating habits questionnaire		x				
Clinical examination:						
- Height (m)		x				
- Weight (kg)		x			x	x
- Blood pressure (mmHg)		x			x	x
- Psoriasis Area Severity Index		x			x	x
- SPARCC Enthesitis Score		x			x	x
- Swollen joint count (66)		x			x	x
- Tender joint count (68)		x			x	x
- Doctors global (VAS 0-100 mm)		x			x	x
- BASMI		x			x	x
- Tender point count		x			x	x
Interview (AEs)				x	x	x
Blood sample analysis:						
- C-reactive protein (mg/L)		x		x	x	x
- Orosomucoid (g/L)		x		x	x	x
- Calprotectin		x		x	x	x
- 1,25-dihydroxyvitamin D (nmol/L)		x		x	x	x
- TSH (miu/L)		x				x
- Hgb (mmol/L)		x				x
- Triglyceride (mmol/L)		x				x
- LDL-cholesterol (mmol/L)		x				x
- HDL-cholesterol (mmol/L)		x				x
- Total-cholesterol (mmol/L)		x				x
- HbA_1C (mmol/mol)		x				x
- HLA-B27 status (+/-)		x				
- Serology tests for *Yersinia, Campylobacter*, *Salmonella* (+/-)		x				
Faecal calprotectin		x		x	x	x
Faecal microbiota analysis		x		x	x	x
Sigmoidoscopy and mucosa biopsy		x				x
Stool, blood, and urine samples (biobank)		x		x	x	x
Intestinal permeability test		x				x
Intervention (+/- FMT)		x				
Serious adverse event forms				x		

Table 1. Protocol schedule of forms and procedures.

ETHICS AND DISSEMINATION

This study is designed as a proof-of-concept clinical trial and will be performed in agreement with GCP-standards, and in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration (64th, 2013). The relevance of the study, the design and the recruitment strategy were evaluated with three patient research partners (PRPs), and alterations especially in primary outcome and recruitment strategy were embedded. Furthermore, a minimum of two PRPs (participating in the study) will be involved in the discussion regarding the progress of the recruitment phase and results, and will be offered the opportunity to comment on the manuscript draft. The Regional Committees on Health Research Ethics for Southern Denmark (DK-S-20150080) and the Danish Data Protection Agency (15/41684) have approved the study protocol. The trial has been registered with ClinicalTrials.gov (NCT03058900) and important protocol modifications will be updated here. The Danish Health and Medicines Authority does not classify the FMT procedure as a medical intervention, and has had no objection to the use of FMT for this study and patient category. Thus, no GCP auditing is legally required. A report describing any potential side effects and adverse events will be submitted to the Ethics Committee yearly. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the Ethics Committee within seven days. Based on these reports, the Ethics committee can determine to terminate the trial early. The Danish Patient Compensation Association provides compensations for patients injured in connection to medical clinical trials.

Although the Danish Health Authorities, for the time being, do not classify donor faecal microbiota as tissue, all steps of the stool donor recruitment, stool donation and FMT preparation will be in accordance with the Danish Tissue Law to ensure that the quality and safety standards laid down in the Danish Legislation BEK nr 764 of May 26, 2015 (implementing Directive 2004/23/EC) are met. Four stool donors will be recruited from the South Danish Transfusion Service & Tissue Centre, Department of Clinical Immunology, Odense University Hospital, and they will be carefully screened for potentially transmissible infections and other conditions associated with gut microbiota function before their stool can be released for FMT. Being a stool donor is voluntary, and no compensation fee will be given. Furthermore, to ensure donor traceability, each patient in the active treatment arm will only receive microbiota from one donor. Also, frozen samples will be clearly labelled with a unique donation code based on the ISBT 128 coding and labelling system, and the release of the final product will adhere to the standards for tissue and blood donation.

Due to the well-documented risk of permanent joint destruction and occurrence of extra-articular manifestations in the PsA disease course, identification of new treatment modalities and biomarkers is essential to help the physician to slow down the disease development or ultimately to prevent it. All PsA patients participating in this study have significant activity in their joint disease despite treatment with the current guideline treatment and first-line drug, MTX, for this condition. This patient population will therefore benefit greatly from new treatment options. Consequently, when weighing the pros and cons, this trial should be performed from a scientific and ethical perspective.

Dissemination will occur through presentations at national and international conferences and publications in international peer-reviewed journal(s).

DISCUSSION

Recent years have seen growing recognition of the complexity of the role of the microbiota in shaping the immune system and its potential effects for health and disease.[22,100,101] In particular, the gut bacteria composition has been associated with the pathogenesis of autoimmune and inflammatory diseases.[102-105] Intriguingly, an abnormal intestinal bacteria composition has been observed in PsA patients, and this association has fostered theories linking intestinal dysbiosis and PsA joint inflammation.[106] Still, it remains to be elucidated whether the intestinal dysbiosis and rheumatic diseases are causal related,[55] and if so, whether dysbiosis is an inciting event in the inflammatory process or a consequence of local and/or systemic inflammation.[54,107] We expect that this double-blind, randomised, placebo-controlled trial will shed new light on this highly relevant topic.

This is the first time that the efficacy and safety of FMT is being investigated in MTX immunosuppressed patients with rheumatic diseases. Subsequently, no data on the feasibility of conducting FMTs in the rheumatological setting is, so far, available. Nor do we know whether one FMT will be sufficient, or whether it should be repeated shortly after the first intervention to normalise the alterations of the intestinal microbiota, which would expectedly enhance any potential anti-inflammatory effects. In the present proof-of-concept clinical trial, the FMT procedure is considered an add-on to the current guideline intervention and first-line drug, MTX. Therefore, from a pragmatic and ethical perspective, we have decided to perform only one FMT (or sham procedure) in each patient even if we are well aware that this approach may not be adequate to achieve long-lasting effects. Indeed, in patients with inflammatory bowel diseases it appears that performing a frequent dosing-regime repeating the FMT procedure up to five times a week for eight weeks provided the best results.[51,108,109] Hence, in contrast to the treatment of *C. difficile* infections where the microbiota is pushed past the point of homeostasis and can be restored following only one FMT,[47] the chronic nature of PsA and other autoimmune and inflammatory diseases, and the somewhat lesser degree of intestinal dysbiosis, may make the host microbiota more resistant to long-lasting modifications. Nevertheless, we hope that the FMT procedure in the present study will be sufficient to boost the effects of MTX so that the participants who are all MTX-non-responders prior to study enrolment will achieve disease control without needing to add or switch to other non-MTX medication.

In the present trial, the primary outcome measure is defined as the occurrence of treatment failure according to shared decision making between patient and physician evaluated at 6 months following the randomised intervention (FMT versus sham procedure). Shared decision making is a process in which both patient and health professional make a decision, taking into account the best evidence of available treatment options and the patient's values and preferences. This approach is considered a key element in the management of rheumatic diseases.[110] As both patients and the treating rheumatologists are blinded to the randomised intervention, the shared decision making will be unaffected by the type of transplant suspension (active or placebo) installed at baseline. Nevertheless, we acknowledge that our assumption that twice as many PsA patients in the sham group will be treatment failures is ambitious, and that we might miss a smaller and less clinically significant treatment effect of the FMT-procedure. In this

case, we hope that our secondary outcome measures will be able to detect potential trends of positive effects in PsA subdomains such as enthesitis score, dactylitis count, and PASI skin score. In addition to the primary endpoint evaluation at 6 months, patients will be asked to fill out a weekly questionnaire regarding side effects as well as skin and arthritis symptoms during the first month following the randomised intervention to reveal any short-term effects on patient-reported outcomes.

Next, only patients with active peripheral PsA will be included. One reason for this is that this will be the first time that FMT is performed on rheumatic patients. Therefore, it seems reasonable only to enrol patients who have had inadequate effect from the initial guideline treatment (MTX), and consequently, on an individual basis could benefit the most from participating in new experimental clinical trials. Also, since patients need to have at least three swollen joints, we expect that we will be able to detect treatment effects of clinical importance. The fact that we do not include recent onset treatment naive patients will, of course, limit our ability to generalise our findings unto the entire PsA patient population. Indeed, in a recent randomised controlled trial of FMT in ulcerative colitis patients, participants with a recent diagnosis (< 1 year) were statistically significantly more likely to respond to FMT compared with those with longer disease duration.[108] That patients will have to subcutaneously administer MTX for at least three months prior to study enrolment will ensure that low intestinal MTX absorption is excluded as a potential effect modifier for the poor MTX response. In addition, as many drugs, including MTX, seem to affect the intestinal microbiological millieu,[111-114] bypassing the intestine during MTX administration will ensure that no local non-disease related effects on the intestinal microbiota will occur.

A great challenge when conducting a trial of FMT is that for the present being there is a lack of both national and international recommendations guiding the regulation and the best clinical practices for donor screening, stool sample handling and preparation of the FMT suspension.[115-117] Indeed, the variability in faecal bacterial communities can complicate or undermine treatment efficacy. This variability stems from both biological variation and variation introduced by sample handling. A recent study reported that oxygen exposure degraded faecal bacterial communities, whereas freeze-thaw cycles and lag time between donor defecation and transplant preparation had much more limited effects.[118] Given that many intestinal bacteria are obligate anaerobe, including many beneficial bacteria potentially possessing anti-inflammatory effects, exposure to oxygen during the preparation of FMT may potentially compromise the therapeutic value of FMT in PsA and other inflammatory diseases. Therefore, although frozen faecal preparations of stool suspended into physiological saline and glycerol have proven just as effective as fresh stool in treating *C. difficile* infections,[119] the optimal transplant preparation method in treating inflammatory diseases remains to be established.

Our stool handling setup is in line with the prevailing practice, which includes mixing and filtration of the stool suspension and adding saline and glycerol as a cryopreservative before storage at -80 ºC.[117] In addition, we have sought to limit the oxygen exposure during transport by placing the donor stool within a plastic bag, which is subsequently put into a tightly closed small plastic container. Supplementary, during preparation the solution will not be homogenized for more than 10 seconds. Nevertheless, the lack of optimal anaerobe conditions during stool

handling could possibly undermine the therapeutic potential of our FMT procedure. Furthermore, although we aim to use 50 g of faeces for each transplant, we acknowledge that the exact weight between donations could vary with an estimated +/- 5 g. Also, due to the wide variability in microbial content in stool between donations, the content cannot be fully standardized, and may likely differ between each FMT procedure. However, to meet this challenge we will collect and store samples from each donation which will enable us to determine the microbiota composition of each donation in case some donations prove more effective than others.

Stool donor selection is another critical issue that needs to be addressed. The composition of the normal microbiota composition has only recently been mapped,[120] and the existence of a limited number of well-balanced host–microbial symbiotic states, where one or more bacteria species are considered the main functional driver(s), have been identified using clustering of metagenomic sequences.[121] Still, the most favourable donor microbiota composition for treating inflammatory diseases has yet to be determined. Therefore, it also remains to be established whether donors with a high stool bacteria diversity should be preferred over isolation of specific bacteria, or if pooled stool samples from several donors outperforms a single-donor transplant.[51,122] We have chosen to use only single donations from four different anonymous stool donors to ensure donor traceability and to enable us to identify any individual donor-specific microbial effects. Also, since host intrinsic-, environmental-, and dietary factors as well as pharmaceutical drugs have been associated with gut bacteria composition and functionality,[111,112,123,124] the donors must eat a balanced diet, not be overweight or take any medications or be physical or psychological stressed, smoke or consume alcohol during the donation period to limit the risk of transferring "abnormal" microbiota to the recipients. These donor criteria have been set for safety reasons, and we acknowledge, that this could potentially limit the inter-donor microbiota diversity due to shared lifestyle characteristics.

Another factor to keep in mind is the concept of matching donor and recipient, which may be of importance for enhancing the colonisation capabilities of the donor microbial communities. In fact, Rossen et al[109] did find that in patients with ulcerative colitis, the microbiota of FMT responders shifted to their respective donors, whereas non-responders did not. Li et al[125] reported that donor bacteria strains established extensively in the recipient and persisted for at least 3 months with a negligible decline of donor-strain populations detected between 45 days and 3 months following FMT in metabolic syndrome patients. However, they also found that recipients receiving the same donor transplant displayed varying degrees of microbiota transfer, indicating individual patterns of microbiome resistance and donor-recipient compatibilities. In addition, host genetics is known to effect the gut microbiota,[126] and animal models have shown that sex[127] and age[128] also can be potentially modifiers of the gut bacteria composition. These observations may prove to be of importance for the outcome of FMT in inflammatory diseases.[129] However, whether sex- and/or age-matching between donor and recipient is crucial for a successful FMT in humans remains to be enlighten. Therefore, in the present study, no donor-recipient matching will be conducted. However, a subgroup analysis will be performed to reveal any trend that could indicate better results in sex- or age-match cases.

Furthermore, as the interactions between the microbiota and the host are influenced by cooperation and competition between pathogenic and commensal microbes and multiple

environmental variables, the lifestyle of the recipient following the FMT may be of importance. Nevertheless, due to the uncertainty of how to define the optimal lifestyle and the lack of knowledge on how different lifestyle factors may interfere with the microbiota, we have decided that the patients in the present study will not have to adhere to any predefined lifestyle "regime" or diet following the randomised intervention. However, every participant will fulfil an eating habit questionnaire at the beginning of the trial.

Finally, non-bacteria microorganisms such as bacteriophages, viruses and fungi may also be of importance when targeting components of the microbiota or host cells for therapeutic purposes.[130-132] Other complicating factors may include the composition of other microbiological niches such as the oral, lung, genitourinary, and skin microbiota.[133,134] Indeed, the latter could likely prove to be of significance in patients with skin psoriasis. However, these factors will not be assessed in the present study.

In conclusion, this trial has the potential to substantially expand the growing body of literature on the role of the intestinal microbiota in general and PsA in particular. Thereby we anticipate that this study will enhance our understanding of cause and effect. The results of this study, when completed, may be exploited for biomarker discovery, and for diagnostic and therapeutic purposes.

AUTHORS' CONTRIBUTION

T. Ellingsen, M.S. Kragsnaes, R. Christensen, and J. Kjeldsen conceived and developed the idea for the study. T. Ellingsen and M.S. Kragsnaes are the principal investigators and wrote the first study protocol draft. T. Ellingsen and M.S. Kragsnaes were responsible for all communication with the scientific ethical committee, the Danish Data Protection Agency and the National Health Board. T Ellingsen is the responsible party and sponsor. M.S. Kragsnaes, T. Ellingsen, H.C. Horn, J.K. Pedersen, H.L. Munk, and U. Fredberg sat up the inclusion and exclusion criteria for the psoriatic arthritis patients, and the latter five rheumatologists are conducting the clinical examinations. J. Kjeldsen, F.M. Pedersen, and H. Glerup discussed and planned the FMT procedure, and they are conducting the FMTs and the sigmoidoscopies (obtaining mucosae tissue samples). D.K. Holm and H.M. Holt helped set up the donor screening programme, and they were responsible for conducting this programme and performing the microbiological and immunological tests. V. Andersen and K. Kristiansen are responsible for the omics and microbiome analyses, and have advised on how the tissue collection should be performed and what kind of tissue would be relevant to collect. R. Christensen has written the statistical analysis plan and will be responsible for the final statistical analyses. In conclusion, all participants designated as authors have contributed to the conception and design of the study, and they have critically either drafted or revised the first draft of the study protocol and the protocol paper. Also, all authors have approved the final version before submission.

REGISTRATION

The trial has been registered with ClinicalTrials.gov (NCT03058900).

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COMPETING INTEREST STATEMENT

None of the team members of this research project has declared any potential conflict of interest.

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FIGURE LEGENDS

Figure 1. Flow diagram of the randomised, placebo-controlled trial.

Figure 2. Participation timeline and characteristics of each visit.

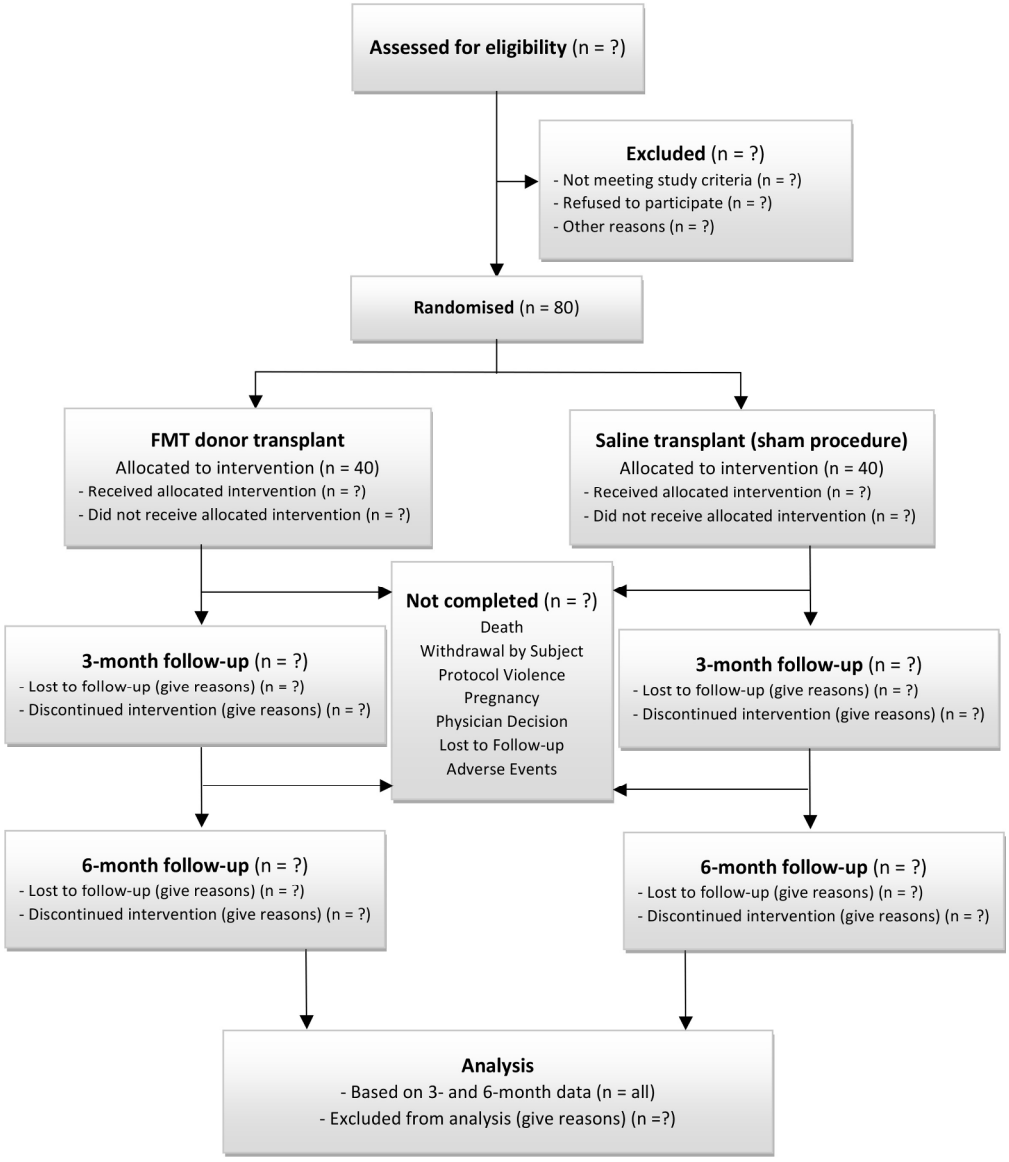

Figure 1. Flow diagram of the randomised, placebo-controlled trial.

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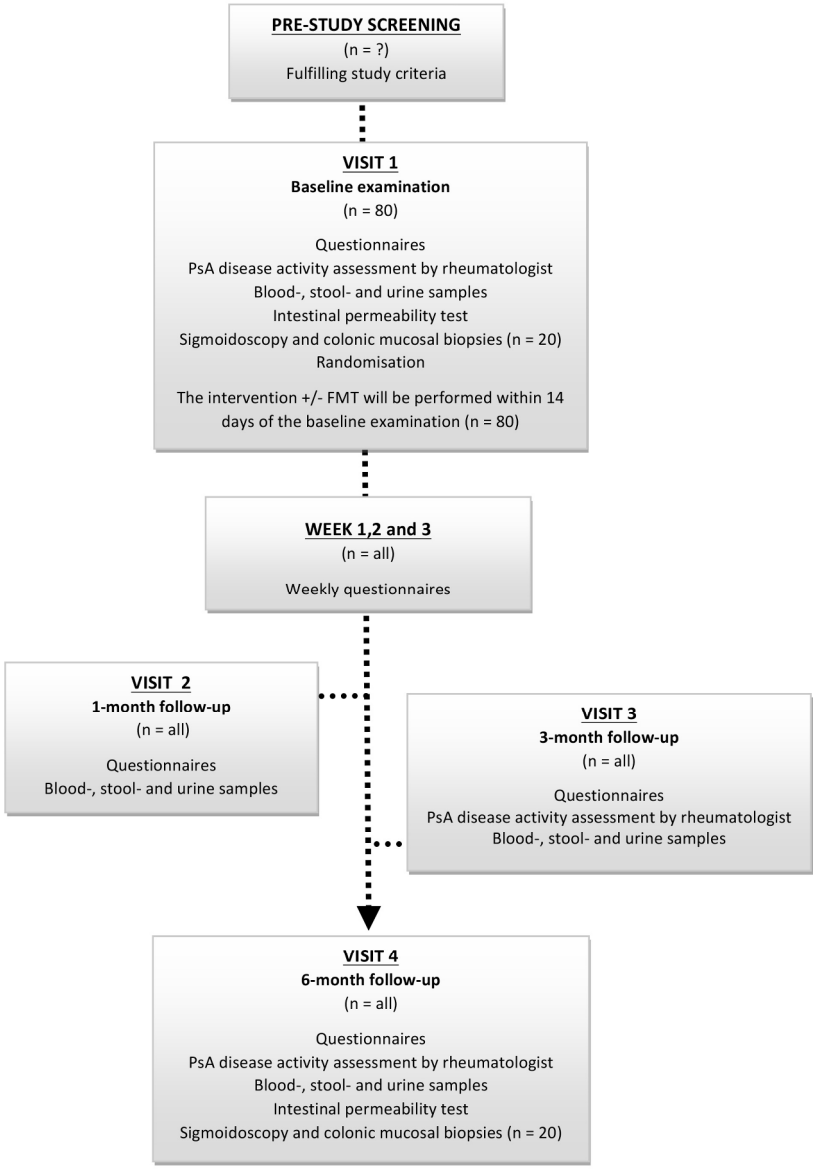

Figure 2. Participation timeline and characteristics of each visit.

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	1-23
Protocol version	3	Date and version identifier	1
Funding	4	Sources and types of financial, material, and other support	23
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1 and 22
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	22

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
	6b	Explanation for choice of comparators	4
Objectives	7	Specific objectives or hypotheses	4-5
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Not applicable
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8 and 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11-12
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	13-14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	14
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	14
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	14
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	14
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	14
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	14
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	18
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	18
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	18
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	18

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	14
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Not applicable
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	18
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	22
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not applicable
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Appendix
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10-11

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.