Research protocol for VEK

Oral Fecal Microbiome Transplantation in Patients with Peripheral Psoriatic Arthritis: A 6-months Randomized, Placebo-Controlled Trial

The FLORA Trial

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ABSTRACT

**Aim:** The main objective is to examine whether oral fecal microbiota donor transplant (OFMT) is more effective than placebo (saline) in reducing disease activity in psoriatic arthritis (PsA) patients with active peripheral arthritis treated with weekly subcutaneously administered methotrexate (MTX).

**Methods/Design:** This is a parallel group double-blind randomized placebo-controlled trial of OFMT combined with subcutaneously administered MTX conducted in two outpatient clinics of rheumatology. Eighty patients with PsA with a minimum of three tender and three swollen joints despite at least three months of MTX treatment (15–25 mg/week) fulfilling the inclusion criteria will be offered participation. The study will run from 2015 to 2020. Clinical measures of PsA disease activity will include plasma C-reactive protein, PASI skin score, SPARCC enthesitis score, swollen/tender joint count (66/68), tender point count, health assessment questionnaire, and visual analog scales of pain, fatigue, patient and physicians global. Side effects as well as adverse events will be registered throughout the study. Participants will be randomized into two groups with an allocation ratio of active to placebo treatment being 1:1. One group (n = 40) will be treated with OFMT from a healthy non-related donor, while the other group (n = 40) will receive a placebo (saline) transplant. Both groups will concomitantly be treated with weekly subcutaneously self-administered MTX (remaining on the pre-inclusion dosage being 15-25 mg/week). Patients and outcome assessors will be blinded for treatment. The primary outcome measure at the 6-months follow-up is the proportion of patients in each group who experience a treatment failure according to shared decision making between patient and physician defined as one of the following: Need for more than one intra-articular injection of glucocorticoid (during the trial period), change to oral leflunomide or sulfasalazine or ciclosporin, or starting biological treatment according to the updated Danish national guidelines due to the severity of the disease activity. Secondary outcome measures are changes in SPARCC enthesitis score, ACR20, ACR50, ACR70, PsARC composite score, and changes in PASI skin score after 3- and 6-months. The degree of intestinal inflammation will be assessed at baseline and at 3- and 6-months follow-up using plasma orosomucoid, fecal calprotectin, analysis of fecal microbiome composition, metabolism and proteomics, in addition to a sigmoidoscopy performed in a subgroup of patients (n = 20) where colonic mucosal biopsy specimens will be obtained. The intestinal permeability will be estimated using translocation markers (mannitol and lactulose) at baseline and at 6-months follow-up. Venous blood will be analysed at baseline, and at 3- and 6-months follow-up for levels of systemic inflammatory markers and cytokine profiles. Tertiary outcome measures are the proportion of patients in each group achieving changes in levels of plasma orosomucoid, fecal and plasma calprotectin, and D-vitamin, changes in levels of specific blood cytokines, macro- and microscopic inflammatory changes of the colonic mucosa after 6-months, changes in the intestinal bacterial metabolism, and changes in risk markers for cardiovascular disease at 6-months. A plasma and serum bank will be established at inclusion of the study for the hopeful development of later validated biochemical markers for disease severity and treatment response. The study is considered a phase 2b clinical trial and will be performed in agreement with GCP-standards.

**Conclusion:** The gut microbiome may be the conductor, or at least a mediator, of the common inflammatory pathways seen in PsA. This study will explore clinical aspects associated with modifying the intestinal flora using fecal donor microbiota.
INTRODUCTION
Psoriatic arthritis (PsA) is a distinct, multi-faceted inflammatory disease with a diverse clinical spectrum and varied disease course. The clinical manifestations include peripheral arthritis, enthesitis and/or spondylitis combined with more or less severe psoriatic skin involvement, nail psoriasis, and/or dactylitis. More than 10% of patients with psoriasis develop PsA during their lifetime. In most patients the symptoms of skin psoriasis appear several years before the arthritic manifestations, however, in around 15%, the musculoskeletal involvement precedes the skin affection. Nearly half of the patients with both early and established PsA also have extra-articular manifestations which can include bowel (16%), ocular, cardiovascular or urogenital involvement. Due to these diverse clinical presentations and the lack of validated biomarkers, diagnosing PsA can be challenging. However, in recent years, the CASPAR classification criteria have been widely adopted (Table 1). This classification system has proven to be especially useful in clinical intervention studies due to its high specificity (nearly 100 percent) reported in populations of both early and established PsA while still maintaining a sensitivity of 87% to 91%. Using the CASPAR classification criteria, the prevalence of PsA among Caucasians is reported to lie between 0.1% and 0.2% and the incidence about 6/100 000 person-years.

Without disease modifying intervention, 40% to 60% of patients will develop erosive and deforming joint damage within a few years of disease onset. Most drug therapy in PsA has not been targeted this specific disease entity but has instead been extrapolated from drugs used to manage skin psoriasis or rheumatoid arthritis. In addition, the intervention strategy of PsA is influenced by many factors including the degree of skin involvement, the location and severity of inflammatory articular involvement, as well as comorbidity and drug toxicity. As the treatment responses seem to differ substantially depending on PsA phenotypes, patients are traditionally grouped into either having predominately peripheral arthritis or axial disease. In axial disease, biological drugs are widely accepted to be the only intervention with acceptable clinical effects when Non-Steroid-Anti-Inflammatory (NSAID) treatment is not sufficient. In peripheral disease, non-biological disease modifying anti-rheumatic drugs (DMARDs), in particular methotrexate (MTX), has long been the mainstay drug for starting therapy. However, only few randomized controlled trials have been conducted, and the most recent study comparing oral MTX 15 mg/week with placebo found no evidence of MTX improving synovitis though it significantly reduced the patient and assessor global scores and skin scores at 6 months. One explanation for this lack of disease modifying effect may be that the absorption of MTX varies widely among patients. Indeed, when comparing the bioavailability across commonly prescribed doses (10, 15, 20, 25mg/week) of oral and subcutaneously administered MTX, there was consistently greater bioavailability of subcutaneous MTX compared with oral MTX administration at all dose levels, with no differences in tolerance and safety measures. Whether this variance can be attributed to changes in the intestinal microbiome and/or subclinical bowel inflammation has yet to be clarified.

PsA pathophysiology and the role of the intestinal microbiome
Although it is well recognized that PsA is a systemic inflammatory disease, limited data exist on the true pathophysiological mechanisms, cellular as well as non-cellular, underlying its heterogeneous disease course. The traditional 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 autoimmune responses involving T-lymphocytes. In particular the Th17 T-lymphocyte population, are likely involved
in the progression of disease, but innate immunity-associated cytokines such as TNF-α are also critically important, as testified by the success of anti-TNF-α strategies. Microbial agents including dormant bacteria, bacteria products, mycobacteria, and viral antigens have all been implicated as the initiators of the immunological cascade. However, no common auto-antigen has yet been identified at the different sites of the PsA disease manifestations. Also, whether the prolonged immune dysregulation as seen in PsA is due to sustained subclinical infections such as micro-abscesses and/or is due to molecular mimicry remains undetermined. While the initiation of skin psoriasis has been associated with an abnormal response to bacteria in the skin due to genetic factors, there has been an increasing interest in how the gut microbiome may be the conductor, or at least a mediator, of the common inflammatory pathways seen in PsA. Indeed, in genetically predisposed patients reactive arthritis, which share some of the clinical manifestations of PsA, can be triggered by certain types of bacterial gut infections such as *Salmonella typhimurium*, *Yersinia enterocolitica*, *Shigella*, and *Campylobacter jejuni*. Also, intestinal dysbiosis has been associated with both rheumatoid arthritis and systemic lupus erythematosus. Intriguingly, a recent study has reported that several intestinal bacteria, including *Akkermansia* and *Ruminococcus*, which are known to play an important role in maintaining gut homeostasis, were practically absent in PsA patients. A similar dysbiosis of diminished *Akkermansia* and *Ruminococcus* species, has been observed in the majority of patients with chronic inflammatory bowel disease. Indeed, changes in the gut bacteria activity (i.e. metabolics/proteomics) may adversely affect the immune system. Still, whether the intestinal dysbiosis is causative or a consequence of systemic inflammation remains to be elucidated.

Mechanisms through which the microbiota may be involved in the pathogenesis of PsA include altered epithelial and mucosal permeability thus compromising the capacity of the intestine to provide adequate containment of luminal microorganisms and molecules, loss of immune tolerance to components of the indigenous microbiota, and trafficking of both activated immune cells and antigenic material to the joints. Indeed, PsA patients show an increase in intestinal lumen IgA relative to healthy controls, and several studies have reported the presence of intestinal inflammation in psoriatic arthritis. One of these research groups observed increased numbers of mast cells, eosinophil granulocytes, and lymphocytes in the duodenal mucosa of PsA patients. They also found that PsA patients had an increased number of lymphocytes in the duodenal epithelium and in the villi in comparison with patients with skin psoriasis. This is in line with a recent study reporting that nearly half of patients with spondyloarthritis (SpA) had microscopic ileum and colon acute or chronic inflammatory lesions, with no difference in prevalence between patients with peripheral and axial SpA. Although SpA comprises several subtypes of arthritis (including PsA), these findings could indicate that the musculoskeletal symptoms rather than the skin affection in PsA may be associated with subclinical bowel inflammation.

**Detection of intestinal inflammation**

The composition of the intestinal microbiome can be determined using the 16s rRNA gene based analysis protocol, and the diversity of the intestinal bacterial communities can be determined using Simpson’s Reciprocal Index of diversity. The bacterial activity (i.e. metabolism) can be measured performing metabolic and proteomic analysis, including colon mucosa proteome analysis using a state-of-the-art proteomics platform. Endoscopy is widely accepted as the gold standard for
detecting and quantifying bowel inflammation.\textsuperscript{37} This procedure is often complemented by a histological evaluation of colonic mucosa biopsies. Other novel non-invasive imaging techniques are capsule endoscopy\textsuperscript{38} and ultrasonic evaluation of the gastrointestinal tract. In addition, fecal biomarkers such as calprotectin,\textsuperscript{39} lactoferrin, and S100A12 are emerging as valuable markers of intestinal inflammation.\textsuperscript{40} These markers are predominantly derived from neutrophils, are easily detectable in the feces, and are useful tools when screening for intestinal inflammation. Another indication of abnormal gut function is the degree of intestinal translocation which is the passage of molecules or microorganisms through the mucosal barrier and to the bloodstream or lymphatic system.\textsuperscript{41} In normal conditions there is essentially no translocation, but dysbiosis and intestinal inflammation can weaken the mucosal barrier function. The intestinal permeability can be measured using traceable markers including measuring urinary lactulose and mannitol (lactulose/mannitol ratio)\textsuperscript{42,43} or Cr-51 EDTA\textsuperscript{44} after oral challenge.

**Fecal Microbiota Transplantation**

Commensal organisms of the intestine are believed to play an important role in maintaining the health of enterocytes, enhancing immune function, and protecting against invasive pathogens.\textsuperscript{45} The goal of fecal microbiota transplantation (FMT) is to break the cycle of imbalance of the intestinal flora. Intestinal dysbiosis may be due to decreased microbial diversity or an increased abundance of certain pathobionts or opportunistic pathogens.\textsuperscript{46} Based on more than 400 cases reported, and a small, open-label, randomized, controlled trial including 43 patients, FMT has demonstrated >90\% clinical resolution of *Clostridium difficile* infections,\textsuperscript{47,48} and administration via nasogastric tube seem as effective as FMT via colonoscopy.\textsuperscript{49} In addition, one small case series revealed that oral administration of frozen encapsulated fecal material from unrelated donors had a similar overall rate of clinical resolution of diarrhea.\textsuperscript{50} Due to these impressive results, FMT is now being tested as a potential treatment for other gastrointestinal and autoimmune diseases. Recently, a pilot study of 30 patients with chronic bowel inflammation has demonstrated that standardized FMT through mid-gut might be a safe, feasible and efficient rescue therapy for refractory Crohn's.\textsuperscript{51} Overall, FMT is considered safe and only few adverse effects have been reported including belching, mild diarrhoea, and abdominal cramping on the day of the procedure.\textsuperscript{48} Whether stool used for FMT should be considered a drug, a tissue product, or should be given its own classification remains to be settled by both national and international regulatory agencies.\textsuperscript{52}

**PURPOSE**

The main objective is to examine whether treatment with oral fecal microbiota transplantation is more effective than placebo (saline) in reducing disease activity after 6 months in PsA patients with active peripheral arthritis treated with weekly subcutaneously administered MTX.

**HYPOTHESES**

1) Oral fecal microbiota transplantation is more effective than placebo (saline) in reducing PsA disease activity in patients with active peripheral PsA treated with subcutaneously administered MTX

A) Primary outcome: Proportion of patients in each group who experience a treatment failure during the 6 months trial period (i.e., shared decision making between patient and rheumatologist\textsuperscript{53}).
B) Secondary outcome measures: Proportion of patients in each group achieving 1: Changes in SPARCC enthesitis score (subgroup analysis of the patients who at baseline have ≥ 1 inflamed enthesis), 2: ACR20-, ACR50-, ACR70-response, 3: Changes in PsARC composite score, and 4: Changes in PASI skin score. After 3- and 6-months.
C) Tertiary outcome measures: Proportion of patients in each group achieving changes in levels of 1: Plasma orosomucoid, 2: Plasma and fecal calprotectin, 3: Fecal IgA, 4: Plasma D-vitamin, 5: Specific circulating inflammatory markers, 6: Macro- and microscopic inflammatory changes of the colonic mucosa, 7: Changes in the intestinal bacterial metabolism, and 8: Changes in risk markers for cardiovascular disease at 6-months.

2) Oral fecal microbiota transplantation can induce more adverse events compared to placebo (saline) in PsA patients with active peripheral arthritis treated with weekly subcutaneously administered MTX.

3) There are no differences in baseline characteristics (including intestinal permeability and fecal microbiome composition, metabolics and proteomics) between responders vs. non-responders (treatment failure) after 6 months in the
   A) OFMT group
   B) Placebo group

4) In the OFMT group, there are no differences between responders vs. non-responders (treatment failure) after 6-months regarding
   A) Changes in the intestinal permeability from baseline to 6-months follow-up
   B) Changes in the fecal microbiome composition from baseline to 1-, 3- and 6-months follow-up
   C) Changes in fecal metabolism and proteomics

METHODS
Study design
This is a randomized – patient, physician and outcome-assessor blind, placebo-controlled, 6-months trial, which will be followed by an open-label extension trial up to 2 years. Patients will be randomly assigned in a 1:1 ratio to receive OFMT or placebo. Outcome assessment will be based on follow-up by a rheumatologist scheduled to occur after 3 months (secondary end-point evaluation) and 6 months (primary end-point evaluation).

Study settings
Departments of Rheumatology in Odense and Svendborg, Odense University Hospital, Denmark.
Department of Medical Gastroenterology, Odense University Hospital, Denmark.
Diagnostic Centre, Department of Rheumatology and Department of Medical Gastroenterology, Silkeborg Regional Hospital, Denmark.
Department of Clinical Microbiology, Odense University Hospital, Denmark.
Organ Centre, Hospital of Southern Jutland, Denmark.
Participants will be included from two outpatient clinics in Denmark - including Department of Rheumatology, Odense and Svendborg, Odense University Hospital, and Diagnostic Centre, Silkeborg Regional Hospital. Recruitment will begin during 2015 (awaiting approval from the Regional Ethics Committee). The specific study period will run from 2015 to 2020.

Eligibility criteria and recruitment
Patients fulfilling the inclusion criteria (see below) will be offered participation in this research project. They will be given oral and written information about the purpose and procedures of the study before informed consent is obtained. No previous or current treatment with biologics are allowed. No treatment with systemic and/or local intra-articular or peritendinous steroid injections, or non-MTX DMARD treatment, or antibiotics are allowed within three months of inclusion. NSAID's must be paused within 14 days of study inclusion, and throughout the 6-months follow-up period. Patients, who do not wish to participate, will be characterized by sex, age and disease activity enabling a secondary assessment of whether the trial can be considered pragmatic.

PsA patients
From August 2015 (awaiting approval from the Regional Ethics Committee) through 2020, all PsA patients at the outpatient clinics at the Departments of Rheumatology (Odense and Svendborg), Odense University Hospital, and Diagnostic Centre, Silkeborg Regional Hospital will be screened for eligibility. 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 CASPAR criteria (see Table 1).
- Presence of active peripheral arthritis defined as ≥3 swollen and ≥3 tender 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 autoimmune diseases than PsA.
- Clinical suspicion of axial PsA by rheumatologist.
- History of severe MTX toxicity or allergic reactions.
- Previous or current biological treatment.
- Systemic and/or local intra-articular or peritendinous steroid injections within three months of inclusion.
- NSAID's within fourteen days or non-MTX DMARD treatment within three months of inclusion.
- Antibiotics within three months of inclusion.
- Inflammatory bowel disease, celiac disease, food allergy, or other intestinal diseases.
- Current cancer.
- Hepatitis B and C, TB, Chlamydia, HIV or HTLV1/2 positive.
- Pregnant or breastfeeding women.
- Not wishing to participate or not suited for project evaluation.
Table 1. The CASPAR criteria.8

Fecal microbiome (stool) donors
Stool donors must be healthy, and preferably active members of the Danish blood donor corps. A stool donor corps consisting of a maximum of 10 individuals from the Aarhus/Silkeborg area and 10 individuals from the Odense area will be recruited through the Danish website http://www.forsoegsperson.dk/, and through local advertisement at the campus of Odense University, Aarhus University, Silkeborg VIA University college, and at the Blood Banks in Aarhus and Odense. Two donors will be contacted in advance when a stool donation is needed to ensure that at least one stool donation is delivered at the time of the planned OFMT. Only stool from one of the donors will be used per transplantation. Each donor will be paid 300 kr. for each stool donation.

Inclusion criteria:
- Healthy individuals.
- Age 18 to 50.

Exclusion criteria:
- Symptoms of gastroenteritis.
- Fecal findings of Salmonella, Shigella, Escherichia coli O157:H7, Yersinia enterocolitica, Campylobacter, Clostridium difficile toxins A and B, ova and parasites.
- Chlamydia Trachomatis infection
- Fecal findings of Helicobacter pylori
- History of high-risk behaviours including recent tattooing or body piercing, multiple sexual partners, persons with recent international travel to areas at high risk for enteric infections or multiple drug resistant bacteria.
- Hepatitis B and C, TB, HIV or HTLV1/2 positive.
• Chronic gastrointestinal illnesses including Crohn’s disease, ulcerative colitis, irritable bowel syndrome, and celiac disease.
• Malignancy (previous or current) or autoimmune disorders including skin psoriasis and psoriatic arthritis.
• Major gastrointestinal surgery (e.g., gastric bypass, short bowel syndrome).
• Severe obesity defined as BMI > 35kg/m².
• Pregnancy.
• Antibiotics, chemotherapy, immunosuppressive drugs (including systemic corticosteroid) within 3 months of donation.
• Hospitalized within 3 months of donation.
• Intake of NSAIDs within 14 days of donation.
• Average alcohol intake more than 7 (women) or 14 (men) units per week.
• Alcohol intake within a week of donation.
• Unbalanced diet or extreme low or high calorie diets.
• Stressful life period.

Donor pre-screening process
Before joining the stool donor corps, each potential donor will be informed about the project and will have to fulfill a consent form. Then he/she must pass a screening process including stool analysis for enteric pathogen (Salmonella, Shigella, Escherichia coli O157:H7, Yersinia enterocolitica, Campylobacter, Clostridium difficile toxins A and B, ova and parasites), Helicobacter Pylori antigen stool test, and blood analysis for HIV, HTLV1/2, hepatitis (A, B and C) using PCR methods, fecal and urine test for Chlamydia Trachomatis using PCR methods, and Tuberculosis (TB) test using a quantiFERON test.

Interventions
Overall study interventions
The OFMT 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 weekly MTX treatment (subcutaneously administered) throughout the study, and they will remain on the same individual dosage which they received at time of study inclusion (a minimum of 15 mg/week cf. patient inclusion criteria) in addition to folic acid supplement on a non-MTX day. Pain killers such as paracetamol and tramadol in recommended dosages (cf. the Danish national guidelines: http://www.danskreumatologiskselskab.dk/index.php?id=52) are allowed, but no NSAIDs can be taken.

OFMT procedure
Patients will be randomized into two groups with an allocation ratio of active to placebo treatment being 1:1. One group (n = 40) will have an OFMT with healthy donor feces, while the other group (n = 40) will be treated with a placebo transplant (brown coloured sterile saline). The OFMT will take place within 14 days of the baseline clinical examination. The evening prior to the OFMT, patients will have to take one dose of oral proton-pump inhibitor. They will meet at the Department of Medical Gastroenterology (Odense or Silkeborg) after an over-night fast (water without sugar or carbon dioxide is allowed). Before the OFMT, a pre-test urine sample will be collected to detect any endogenous
mannitol (for the intestinal permeability test). Donor feces should be fresh, i.e. not older than 6 h, and stored in airtight vessels at +5°C to +8°C to avoid bacterial overgrowth. The OFMT transplant will consist of 60 g of stool sieved to remove particulate material, followed by suspension in 100 mL sterile saline (0.9% NaCl). The homogenized and filtered suspension will be apportioned into a 60 mL syringe, and processed rapidly, to optimize the protection of anaerobic bacteria from oxygen. A total of 120 mL suspension will be installed in the duodenum using an oral-duodenal tube followed by non-bacteriostatic saline flush. The correct placement of the tube will be confirmed using gastroscopic guidance.

To examine the intestinal permeability, a sugar-test solution of 100 mL containing 5 g of mannitol and 10 g of lactulose in water will be installed following the OFMT. The patients will then have to continue fasting for 2 h, and urine will be collected for 5 h. During this period, the patient will remain admitted at the department for observation.

Treatment strategy for OFMT non-responders
Patients who during follow-up present with increased disease activity, will, depending on the clinical presentation, be offered another treatment strategy which may include local intra-articular steroid injections, change to another non-MTX DMARD, or biological treatment. If the patient accept such treatment changes, this will be characterized as OFMT treatment failure according to the primary outcome definition (one intra-articular steroid injection is allowed). This strategy ensures that all patient will be treated in accordance with current Danish guidelines for PsA meaning that all the included patients will receive guideline intervention strategy (http://www.danskreumatologiskselskab.dk/index.php?id=52).

MTX toxicity and drop-outs
Blood tests for MTX toxicity will be performed in accordance with current clinical practice. The frequency of these routine blood test will be decided by the responsible treating rheumatologist depending on symptoms or signs of MTX toxicity. Therefore, the exact number of tests and the precise timeline for performing these tests may deviate from the one suggested in figure 2. In case of MTX toxicity, severe side effects, pregnancy, or occurrence of infectious disease or other diseases which contraindicate MTX treatment, MTX dosage will be decreased or the treatment will be paused. These patients will remain in the study (unless there condition contraindicate this), and they will be analysed as members of the treatment group to which they were randomized using intention-to-treat-type analyses.

Randomization
We will perform randomization according to a parallel-group, placebo-controlled trial to explore the efficacy and safety. Patients will be randomly allocated to receive either fecal microbiota donor transplant or placebo transplant. The participants, investigators (other than the gastroenterologists performing the transplant procedure), will remain unaware of the group assignments. Eligible participants will be randomly assigned in permuted blocks of 4 and 6, according to computer-generated random numbers, to undergo either OFMT or a saline (sham) procedure. Participants will be stratified according to center. To ensure concealment of the assigned intervention, the treating gastroenterologist obtain the opaque, sealed envelope containing the participant’s assigned
intervention from the site’s receptionist just before the procedure is performed. Only the receptionist had access to the site’s assignment schedule. Neither the receptionist nor the treating gastroenterologist will have any other role in the data analysis. The patient and outcome assessor will be blinded to the treatment.

**Figure 1. RCT flow diagram**

![RCT Flow Diagram]

- **Assessed for eligibility (n = ?)**
  - Excluded (n = ?)
    - Not meeting inclusion criteria (n = ?)
    - Refused to participate (n = ?)
    - Other reasons (n = ?)
  - Randomized (n = 80)
    - Fecal microbiota donor transplant
      - Allocated to intervention (n = 40)
        - Received allocated intervention (n = ?)
        - Did not receive allocated intervention (n = ?)
    - Saline transplant (placebo)
      - Allocated to intervention (n = 40)
        - Received allocated intervention (n = ?)
        - Did not receive allocated intervention (n = ?)

- 3-months follow-up
  - Loss to follow-up (n = ?)
    - Discontinued due to
      - Adverse events (n = ?)
      - Worse disease (n = ?)
      - Patient choice (n = ?)
  - 6-months follow-up
    - Loss to follow-up (n = ?)
    - Discontinued due to
      - Adverse events (n = ?)
      - Worse disease (n = ?)
      - Patient choice (n = ?)

- Analysis
  - Based on 3- and 6-months data (n = ?)
Baseline variables and outcomes

At baseline, the participants will be demographically characterized by gender (male/female), age (years), height (m), body weight (kg), smoking status, duration of skin psoriasis (years), duration of arthritis (months), and previous anti-inflammatory treatment including the use of oral NSAIDs. Presence of gastrointestinal symptoms including abdominal pain (0 = none, 1 = mild, 2 = moderate, 3 = severe), number of stools per 24 hours, blood or mucus in the stool (yes/no), and number of daily vomiting for the last week. Also, HLA-B27 status and serology tests for Yersinia, Campylobacter, Salmonella, Shigella, and Chlamydia trachomatis, as well as urine test (morning urine, 20-30 mL) and fecal tests for Chlamydia Trachomatis, and Helicobacter Pylori antigen stool test on 10 mL stool (pos/neg) will be performed. All variables are listed in table 2.

Disease activity will be scored by the use of number of swollen joints (66) and number of tender joints (68) assessed by a rheumatologist, plasma C-reactive protein (CRP) (mg/l), Health Assessment Questionnaire (HAQ) range 0-3 with a higher score indicating higher disability, nail disease (yes/no), dactylitis (20 digits (alternatively: yes/no)), Psoriasis Area Severity Index (PASI), SPARCC Enthesitis Index, BASMI, and tender point count. Visual analog scales (VAS) 0-100 mm (0=best, 100=worst) will be used to assess pain, fatigue, patient and physicians global assessment.

"Intestinal inflammation" will be assessed using fecal calprotectin (15 mL stool), plasma orosomucoid (alpha-1-acid glycoprotein) (g/L), plasma calprotectin, fecal IgA, and by determining the composition of the intestinal microbiome (e.g. Firmicutes/Bacteroidetes ratio) using the 16s rRNA gene based analysis protocol on frozen stool samples collected at baseline, 1-, 3-, and 6-months. In addition, 20 patients will have a flexible sigmoidoscopy performed at baseline and at 6-months follow-up to evaluate macroscopic changes of the colonic mucosa including changes in the normal vascular pattern, and presence of erythema, oedema, granularity, blood in lumen, erosions (small superficial defect in the mucosa), ulcerations (larger and deeper excavated fibrin covered lesions), and friability (bleeding mucosa either at contact or spontaneously). These areas will also be graded using the Mayo endoscopic score. Furthermore, colonic mucosal biopsy specimens preserved in formalin will be evaluated regarding presence of architectural changes and crypt destruction, as well as presence and location (crypt, epithelium, lamina propria) of immune-competent cells including T-lymphocytes (CD3, CD4 and CD8), B-lymphocytes (CD20), neutrophils (granzyme-B), NK cells (CD56), mast cells (tryptase) and eosinophils. The mucosaes metabolics and proteomics will be examined in mucosaes specimens (snap frozen) and similar metabolic and proteomic analysis will be performed on stool, urine and blood at baseline, 1-month, 3-months, and 6-months follow-up. The intestinal permeability will be determined using an oral sugar test (lactulose/mannitol ratio) on the day of the OFMT and at the 6-months follow-up. Blood samples will be analysed for presence of gut-derived bacteria and pathogen associated molecular patterns antigens as a measure of the degree of intestinal translocation (i.e. gut bacteria products passing the intestinal mucosa barrier into the blood stream).

Blood tissues bank: A blood sample of 50 mL for serum and plasma storage will be obtained at inclusion and at 6-months follow-up for analysis of biochemical markers. Immunologic and early diagnostic markers such as connective tissue metabolettes like SPD, MFAP4, COMP as well as M-ficolin, MAP44, and chemokines/cytokines including CCL2 (monocyte chemotactic protein 1, MCP-
CCL19 (macrophage inflammatory protein 3\(\beta\), MIP-3\(\beta\))\(^1\), IL-6, VEGF\(^79\), 14-3-3 protein, and adiponectin\(^80\) will be quantified as potential new biomarkers of PsA severity and treatment response. For the proteomic and metabolic analysis a blood sample of 60 mL (30 mL blood EDTA and 30 mL plasma), an urine sample of 50 mL, and a fecal sample of 5 g will be obtained at baseline, 1-, 3-, and 6-months. Also, in 20 participants 10 mucosae biopsies (each biopsy being 5 x 5 mm (5-9 mg) from the sigmoid colon of each patient will be obtained and snap-frozen in liquid nitrogen at baseline and 6-months follow-up. The tissue bank collected in relation to the project is closely related to the purpose of the present project.

**Follow-up**

1-month: Telephone interview regarding any potential side effects; blood-, urine- and stool sample.
3-months: Clinical examination and interview; stool sample; urine sample; and blood sample.
6-months: Clinical examination and interview; intestinal permeability sugar-test; stool sample; urine sample; blood sample; and sigmoidoscopy and mucosae biopsy (subgroup only).

**Outcomes at follow-up**

**Primary outcome at 6 months (RCT):** Treatment failure according to shared decision making between patient and physician defined as one of the following:
- Need for more than 1 intra-articular glucocorticoid injection given after 3 months due to disease activity.
- Need for change to the updated Danish national guideline treatment\(^1\) of other synthetic DMARDs (at the moment oral leflunomide, sulfasalazin or ciclosporin) due to disease activity.
- Need for biological treatment according to the updated Danish guideline treatment\(^1\) due to severe disease activity.

\(^1\)The Danish national treatment guidelines for PsA: [http://www.danskreumatologiskskelsab.dk/index.php?id=52](http://www.danskreumatologiskskelsab.dk/index.php?id=52)

**Secondary outcomes at 3- and 6 months (RCT):** Proportion of patients in each group achieving ACR20-, ACR50-, ACR70-responses (ACRx%; a composite score including an improvement of at least xx% in swollen and tender joint count (66/68)\(^81\) and in three of five other measures including C-reactive protein level; physician global assessment of disease activity; patient global assessment of disease activity; patient pain assessment; disability (HAQ)\(^58,59\), PsARC composite score (PsARC; a composite measure consisting of improvement in joint swelling or tenderness in association with improvement in any of four other measures (patient global assessment of articular disease; physician global assessment of articular disease; joint pain or tenderness; joint swelling), changes in SPARCC Enthesitis Index,\(^61-63\) and Psoriasis Area Severity Index (PASI).\(^60\)

**Tertiary outcomes at 6 months (RCT):** Proportion of patients in each group achieving changes in levels of specific circulating inflammatory markers, macroscopic changes of the colonic mucosa, microscopic changes of the colonic mucosa, changes in plasma CRP, changes in plasma orosomucoid, changes in plasma and fecal calprotectin, changes in plasma D-vitamin, changes in the intestinal bacteria metabolism and proteomics, changes in cardiovascular risk factors including body Mass Index, blood pressure, plasma triglyceride, plasma LDL-cholesterol, plasma HDL-cholesterol, plasma total-cholesterol, HbA1C, and changes in tender point count.\(^64\)
Occurrence of adverse effects: Mild gastrointestinal side effects (abdominal pain, number of stools per 24 hours, blood or mucus in the stool (yes/no), and number of daily vomiting), other symptoms, which will be registered on a daily basis the first month following the OFMT, as well as severe adverse effects requiring hospitalization throughout the study period.

Intestinal permeability test at 6 months
Changes in the intestinal permeability between baseline and 6-months follow-up in the PsA group (n = 80), and in a sub-group of healthy stool donors (n = ?). The test includes consuming a solution (10 g lactulose and 5 g mannitol in 100 ml of deionised water (675 mmol/kg) in the morning after a 6-hour fast and following the discharge of morning urine. For the next 5 hours, urine will be collected in a specialized container (containing 1mL chlorhexidine 200 g/L as a preservative). The total volume of urine will be recorded and a sample of 10 mL will be aliquoted and stored at -40°C. No drinking or eating are allowed within 2 hours after consuming the solution. Measurement of mannitol and lactulose in urine will be performed using high-performance liquid chromatography.
Routine screening for MTX toxicity will be performed at week 4, 10, 16, 22.

**PRE-STUDY SCREENING**
(n = ?)
- Fulfilling inclusion criteria

**VISIT 1**
Baseline examination (n = 80)
- Questionnaire
- PsA disease activity assessment by rheumatologist (clinical and ultrasonic examination)
- Blood sample
- Stool sample
- Urine sample
- Sigmoidoscopy and colonic mucosal biopsies (n = 20)

S.c. MTX ≥15mg/week (n = 80)
Within 14 days of the baseline examination (n = 80)
Randomization and treatment +/- OFMT
Intestinal permeability test (urine sample)

**VISIT 2**
3-months follow-up examination (n = ?)
- Questionnaire
- PsA disease activity assessment by rheumatologist (clinical and ultrasonic examination, and CRP)
- Stool sample
- Urine sample
- Blood sample

**VISIT or TELEPHONE**
(n = ?)
- Gastrointestinal symptoms (diary)
- Stool, blood and urine samples

**VISIT 3**
6-months follow-up examination (n = ?)
- Questionnaire
- PsA disease activity assessment by rheumatologist (clinical and ultrasonic examination, and CRP)
- Blood sample
- Stool sample
- Urine sample
- Intestinal permeability test (urine sample)
- Sigmoidoscopy and colonic mucosal biopsies (n = ?)

Figure 2. Participant timeline.

[15]
**Protocol schedule of forms and procedures**

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<th>Activity/assessment</th>
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<th>Prestudy screening</th>
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</tbody>
</table>

**Clinical examination:**
- Height (m)                                  | x            | x                  | x                | x       |
- Weight (kg)                                  | x            | x                  | x                | x       |
- Blood pressure (mmHg)                        | x            | x                  |                  |         |
- Psoriasis Area Severity Index                | x            | x                  |                  |         |
- SPARCC Enthesitis Score                      | x            | x                  |                  |         |
- Swollen joint count (66)                     | x            | x                  |                  |         |
- Tender joint count (68)                      | x            | x                  |                  |         |
- Patient global (VAS 0-100 mm)                | x            | x                  |                  |         |
- Patient fatigue (VAS 0-100 mm)               | x            | x                  |                  |         |
- Doctors global (VAS 0-100 mm)                | x            | x                  |                  |         |
- BASMI and BASDI                              | x            | x                  |                  |         |
- Tender point count                           | x            | x                  |                  |         |

**Blood sample analysis:**
- C-reactive protein (mg/L)                    | x            | x                  |                  |         |
- Orosomucoid (g/L)                            | x            | x                  |                  |         |
- Calprotectin                                  | x            |                    |                  |         |
- 1,25-dihydroxyvitamin D (nmol/L)             | x            |                    |                  |         |
- TSH (miu/L)                                  | x            |                    |                  |         |
- Hgb (mmol/L)                                 | x            |                    |                  |         |
- Triglyceride (mmol/L)                        | x            |                    |                  |         |
- LDL-cholesterol (mmol/L)                     | x            |                    |                  |         |
- HDL-cholesterol (mmol/L)                     | x            |                    |                  |         |
- Total-cholesterol (mmol/L)                   | x            |                    |                  |         |
- HbA1C (mmol/mol)                             | x            |                    |                  |         |
- HLA-B27 status (+/-)                         | x            |                    |                  |         |
- Serology tests for *Yersinia*, *Campylobacter*, *Salmonella*, *Shigella*, and *C. trachomatis* | x         |
- Special immunological analysis (biobank: MCP-1, CCL19, IL-6, MaP44, 14-3-3 protein, MFAP4, M-ficolin, COMP, SPD, bacteria products) | x         |

**Urine and fecal *Chlamydia trachomatis* (DNA)**

**Fecal calprotectin**

**Fecal supernatant IgA**

**Fecal microbiome analysis and tests for enteric pathogens**

**Sigmoideoscopy, mucosa biopsy**

**Stool, blood, and urine samples (biobank: Metabolic, proteomic and microbiological analyses)**

**Intestinal permeability test (urine) (optional for stool donors)**

**Oral fecal microbiome transplant**

**Serious adverse event forms**

**Communication log**

**Table 2. Protocol schedule.**
Research plan and responsibilities of each study member

MK is responsible for every participant receives comprehensive oral and written information about the purpose and procedures of the study before signing the consent form. However, as this is a multicenter project, UF, AJ, HM, JP, HH, IH, JK and TE are all authorized to give the same pre-study inclusion information and subsequently obtain the patient's informed consent. Patients will attend the same hospital unit (Odense or Silkeborg) throughout the study. MK, UF, AJ, HM, JP, HH, IH and TE will perform the clinical examinations at baseline and at the 3- and 6-months follow-up (MK will not participate in the clinical evaluation at follow-up). Blood samples will be obtained by a phlebotomist at baseline and at the 1-, 3- and 6-months follow-up, in addition to the routine blood screening for MTX toxicity and levels of CRP. The routine blood analysis will be performed at the Departments of Clinical Biochemistry (Odense and Silkeborg) while the advanced blood analysis for cytokines and connective tissue metabolites as well as proteomics and metabolics will be performed at specialized departments including Organ Centre, Hospital of Southern Jutland, Denmark. and Institute for Molecular Medicine, Southern University of Denmark. Collecting stool- and urine- samples is the responsibility of MK assisted by a study nurse. Fecal analysis, including healthy stool donor screening, is the responsibility of HMH and will be performed at the Department of Clinical Microbiology, Odense University Hospital. Sigmoidoscopies will be performed by HG, OB and JK, and they will also obtain the colonic mucosal biopsy specimens in a subgroup of participants. Only 20 patients will have a sigmoidoscopy, and they will have to give their explicit written consent to participate in this specific procedure. MK will perform the OFMT/placebo treatment allocation. The OFMT procedure will be performed by JK, HG, and OB assisted by MK and a study nurse. The study nurse will also be around the patient before and after the OFMT procedure, and will prepare the transplant solution, assist the gastroscopic guidance of the oral duodenal tube, and help collect urine samples for the intestinal permeability test. VA will perform the metabolic and proteomic analysis on mucosa, stool, urine and blood. Data will be analyzed by MK supervised by TE and RC. MK will draft the first version, which will be commented by the project participants (see under publication).

Data management

All study-related information will be stored securely at the study sites. Laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded ID number (FMT-PsA-xx (patient no), visit type (e.g. baseline, 1-month, 3-months, or 6-months), date), and documents, questionnaires and clinical forms will be dated and signed. Data collection from the clinical and ultrasonic examinations will initially be on original paper based forms followed by electronically data entry at the participating site where the data originated. The original forms will be kept on file at the participating sites, and maintained in storage for a period of three years after completion of the study. 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, and all personal identifiable data, including tissue samples, will be stored according to law. A list of source data with a description of where source data etc. can be found for all study sites will be prepared before the trial is initiated. This source data list will be available in the Investigator’s Trial Master File. Authorisation from the Danish Data Protection Agency will be secured prior to the commencement of processing of personal data. Collection of personal data for use in the project will not commence before authorisation has been granted.
ETHICAL CONSIDERATIONS
The procedures followed are in accordance with the ethical standards of the responsible committee on human experimentation (in institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. This study will only include PsA patients with active peripheral joint disease despite a minimum of 3 months of weekly subcutaneously administered MTX treatment (≥15 mg/week), which is the current Danish guideline intervention for this disease presentation. 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.

Involvement of patient representatives in the scientific process
The relevance of the study, the design and the recruitment were evaluated with selected patients, and alterations in especially primary outcome and recruitment strategy were embedded. Two patient partners will be involved in discussion regarding progress of the recruitment phase, results, and offered the opportunity to comment on the draft of the manuscript. All participants will provide written informed consent for the study, and will subsequently be registered with ClinicalTrials.gov.

Recruitment
This research project (FMT-PsA) is a randomized controlled trial conducted in two out-patients clinics of rheumatology with a 3- and 6-months follow-up period. The inclusion period will terminate when the 80th PsA patient has been enrolled, or when the study period ends (2020). All eligible patients will be told that their participation is entirely voluntary, and that they can withdraw from the study for any reason at any time. Also, their final decision will not affect their future treatment at the clinic. In addition, they will be given oral and written information about the purpose and procedures of the study, and will receive the hand out information: “The rights of individuals participating in a biomedical trial”. They will get time to consider, and if choosing to participate, each patient will have to sign the document “Informed consent” before the baseline examination is initiated. No study related procedures will be conducted before written consent. No compensation will be provided for PsA patients’ study participation.

Obtaining informed consent
PsA patients
The oral and written information will be given at the Department of Rheumatology, Odense University Hospital, or Diagnostic Centre, Silkeborg Regional Hospital depending on where the patient is normally followed, when the patient attends a routine control examination after being treated with the national guideline treatment (subcutaneously administered MTX) for at least 3 months. If the patient still presents with severe joint disease (≥ 3 swollen joints and ≥ 3 tender joints), he/she will receive oral and written information about the purpose, procedures and potential risks and complications of the study. The information will be given by either the project leader, MD, Maja Skov Kragsnæs, or by one of the participating rheumatologists (UF, AJ, HM, JP, HH, IH or TE). The conversation will take place in a quiet setting, i.e. in a consultation room where the door can be closed, so the conversation can take place undisturbed. If the patient wishes to bring an assessor, a new date for the orally and written......
information will be scheduled. From the oral and written information has been given and until the informed consent is obtained, the patient will get time to consider. Questions arising in this clarification phase will be answered by phone, mail or in person (after the participant's choice).

**Donors**
Stool donors will be recruited following local advertisement outlining the purpose of the study and the type of donor involvement. If a potential participant is interested, he/she will have to write to the specified email address, and here provide a phone number. Then he/she will be contacted by phone by the project leader, MD, Maja Skov Kragsnæs, and will receive the written participant information. The potential participants will get time to consider, and if he/she remains interested, a meeting will be arranged at either Odense University Hospital, Silkeborg Region Hospital or Aarhus University Hospital (after the donor's wish), where the project leader, MD, Maja Skov Kragsnæs will clarify any questions, provide information about the screening process, and obtain the informed consent. For this interview, the potential donor can bring an assessor.

**Study intervention and change in treatment strategy**
The OFMT will be an add-on strategy for PsA patients with active joint disease despite ongoing treatment with weekly subcutaneously administered MTX. Therefore, throughout the study, patients will be treated with the same MTX dosage which they received at time of study inclusion (a minimum of 15 mg/week cf. patient inclusion criteria) in addition to 5 mg folic acid on a non-MTX day. Patients who during follow-up present with increased disease activity, will, depending on the clinical presentation, be offered another treatment strategy which may include add-on NSAIDs, local intra-articular steroid injections, non-MTX DMARD, or biological treatment. This strategy ensures that all patients will be treated in accordance with current Danish guidelines for PsA meaning that all the included patients will receive guideline intervention strategy (cf. http://www.danskreumatologiskselskab.dk/index.php?id=52).

**Oral fecal microbiome transplantation**
The trial will be conducted in accordance with good clinical practice (GCP-standards), and the data will be registered and reported according to procedures that assure the quality of every aspect of the trial. Healthy stool donors, preferably active blood donors, will only be approved if they do not fulfil any exclusion criteria and if the fecal screening for ova and parasites, *Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli*, and *C. difficile* toxin A and B by polymerase chain reaction (PCR), *Helicobacter Pylori* antigen stool test, as well as the urine screening for *Chlamydia Trachomatis* and serum screening for hepatitis A, B, and C, human immunodeficiency virus (HIV), HTLV1/2, and TB (quantiFERON test) come out negative. By performing these test on all donors, we can minimize the risk of transferring any pathogens from the donor to the recipient.

Based on few RCT's and several reviews of OFMT procedure case reports, OFMT is considered safe and only few adverse effects have been reported including belching, mild diarrhea, and abdominal cramping on the day of the procedure. The long term effects of OFMT remain largely unknown. Any suspected unexpected serious adverse reactions will immediately be reported to the Danish Health and Medicines Authority, and the Regional Ethics Committee. In this study, the OFMT will be performed by one of three experienced senior gastroenterologist assisted by MK and a study
nurse. The fecal microbiome will be administered through an oral tube, and the correct placement of the tube will be confirmed using gastroscopic guidance. Normally, this procedure can be performed with only minimal patient discomfort by applying local throat sedation using an oral spray. If a participant needs more sedation, small amounts of midazolam and/or rapifen can be given intravenously.

**Subcutaneously administered MTX**

MTX comes in two forms: Tablets and injections. Traditionally, tablets have been first choice. In case of severe gastrointestinal side effects following tablet intake, or if the patient suffers from gastrointestinal problems or other comorbidities which might compromise the MTX absorption, subcutaneously administered injection will be preferred. Study participants will be instructed in the injection procedure by trained nurses at the departments of rheumatology. This type of injection is easy to do and besides mild pain at the puncture site, the injection is not associated with severe complications. Nor is it a problem if the MTX accidently goes into the muscle or skin. Participants will be supplied with MTX and all equipment necessary including alcohol swabs, syringes, needles and sharps containers. Vials of MTX should be stored dry at room temperature out of the reach of children.

**Sigmoidoscopy and colonic mucosal biopsy specimens**

To clear the rectum and lower colon for stools, the participant will have to take a laxative prior to the examination. Most sigmoidoscopies are done without any problem. The procedure is not usually painful but it may be a little uncomfortable. The intestinal mucosa biopsy procedure is completely painless. For this reason, sedation is not routinely offered for this examination, but may be offered according to patient wishes. The sigmoidoscopy itself usually takes about 15-20 minutes. Some people have some crampy pains and excess wind after the procedure, and it is not unusual to experience some diarrhoea for a couple of days post procedure until the bowel returns to its normal function. Rarely, the sigmoidoscope may cause damage to the colon. This may cause bleeding, infection, and very rarely, perforation (less than 3 in 10,000). Participants will be informed to consult a doctor immediately if any of the following occur within 48 hours after a sigmoidoscopy: Severe abdominal pain, passing a lot of blood per rectum, or fever.

All sigmoidoscopies performed in relation to this study will be performed by one of three experienced senior gastroenterologists. We believe that examining the distal part of the colon and obtaining mucosa specimens for histological evaluation are an essential part of the study to support the notion of subclinical bowel inflammation in PsA patients, and in order to relate presence of inflammation with the OFMT treatment outcome. However, as the sigmoidoscopy is only related to the secondary and tertiary outcome measures, we have chosen only to perform the procedure in 20 of the 80 participants.

**Donor considerations**

Stool donors will be found following public advertisement. The stool donor will have to answer questions regarding risk behaviour and medical health. Also, a blood sample will be obtained (see the paragraph below) if the stool donor is not already an active blood donor following the strict Danish blood donor regulations and controls, and thereby recently have been screened for transmittable diseases. A laxative (Bisacodyl 10 mg suppositories, Dulcolax®) can be taken by the donor on the
morning of the stool donation. Besides the times used for the screening process, donating stool is a completely safe and painless procedure. Each donor will be paid 300 kr. per stool donation.

In addition, stool donors will be given the opportunity to participate in an intestinal permeability test which will include an overnight fast, and intake of an oral sugar solution followed by 2 hours fasting and 5 hours of urine sampling. This test will require the explicit informed consent of the donor after having received written and oral information about the test procedure. The procedure is completely safe and painless. Participating in the intestinal permeability test is not obligate for being a stool donor, but performing the test in a sub-group of healthy donors at inclusion and after 6 months will provide important information about the function of the intestinal barrier in healthy individuals. All donors who wish to participate in the intestinal permeability test will be included. Each donor who participates in this test (at inclusion and after 6 months) will be paid a total of 600 kr. If a donor leaves the study before the study terminates, proportional payments will be given.

**Blood sampling**

Peripheral venous blood will be obtained at baseline and at the 3- and 6-months follow-up. This is part of the normal standard procedures performed on all PsA patients attending the Department of Rheumatology, Odense University Hospital, or Diagnostic Centre, Silkeborg Regional Hospital. In addition to these routine blood samples, blood samples of 110 mL for EDTA serum and plasma storage will be obtained as part of a biobank at baseline, 1-, 3- and 6-months follow-up. Obtaining this extra samples blood will not cause any additional discomfort for the patient. In relation to the blood sample procedure, mild pain associated with the puncture of the skin might occur, but normally, this will only last for a few seconds. Also, when punctuating the skin, there exist a small risk of developing an infection. This risk is estimated to be less than 1/25,000.

**Establishing a biobank**

All blood-, urine-, stool- and mucosae samples obtained from patients which are not used in the routine assessment will be stored in a biobank with proper identification number for 15 years in order to perform the current analysis of the FMT-PsA study. Remaining stool samples from donors which are not used for transplantation or microbiological evaluation will be destroyed. Tissue samples will be stored in a freezer in a locked room with limited access. On request of any of the participating patients, specific tissue will be destroyed. In addition, the Regional Scientific Ethical Committee will be contacted if any future analysis on the tissue from the biobank extends beyond the present application. After 15 years, all material will be destroyed.

**Follow-up examination**

In order to arrange a date for the 3- and 6-months follow-up examinations, patients will be contacted by letter/email or phone one month prior to examination using the contact information provided by each patient at baseline. Loss to follow-up will be clarified by phone or e-mail, and if possible, the reason for not participating will be clarified. This question will only be asked once, and the patient has the right not to give any answers. In the case of a patient agrees to participate in the follow-up examination, but does not show up at the arranged date, he/she will be contacted once more by phone or e-mail to clarify the reason (drop out or forgetfulness).
Comorbidity
If the clinical examination, blood sample analysis or other examinations or analysis reveal indications of pathological irregularities, the affected patient or stool donor will be referred to the relevant department for further investigation.

Study registrations and approvals
Before the FMT-PsA research project will begin, this study protocol will be registered at ClinicalTrials.gov following the approval by the Regional Ethics Committee, and the Danish Data Protection Agency. The Danish Health and Medicines Authority has already been contacted regarding how this trial should be classified. Their preliminary answer to our oral enquiry has been that FMT is not a medicinal product or a medical device, and therefore this project is not classified as a medical trial that needs approval by this authority. In order to make their final written decision, the Danish Health and Medicines Authority is now reviewing the complete protocol allowing for a quick response. In case this project is subject to a formal notification, the Regional Ethics Committee will immediately receive a copy of the notification front page. However, no matter what the Danish Health and Medicines Authority's final written decision states, this trial will be conducted in accordance with GCP-standards.

Conclusion
The psoriatic arthritis patients participating in this study all have significant activity in their disease despite treatment with the current guideline treatment and first-line drug (MTX) for this condition. This population will therefore benefit greatly from new treatment options, including new interventions with better side effect profiles than those the currently used disease-modifying medications possess. At best, this study could shed new light on why the disease occurs, which may be the first step towards a curative treatment. Donors will not get any direct or indirect benefits of this study's results, but they will be financially compensated for their participation. Besides the time spent, the donor-related procedures (stool donation and intestinal permeability test) are completely safe and pain free. Thus, when weighing the pros and cons of the project, this study should be performed from a scientific and ethical perspective.

STATISTICAL CONSIDERATIONS
We consider the study to be an exploratory (phase 2b) clinical trial. For a comparison of two independent binomial proportions using Pearson’s Chi-square statistic with a Chi-square approximation with a two-sided significance level of 0.05, a sample size of 40 PsA patients per group has a power of 90% (0.895) when the proportions of treatment failures are 35% (active group) and 70% (sham group), respectively. Consequently, the inclusion of 80 PsA patients allocated to two treatment arms is believed to be sufficient to reveal any difference of clinical importance between treatment groups. The study will be performed in agreement with GCP-standards. The statistical analysis will be performed in collaboration with senior biostatistician RC. Data will be analysed with the STATA statistical package (version 12; StataCorp LP), and SAS software (v. 9.3; SAS Institute Inc., Cary, NC, USA).

The full analysis set will consist of all randomized subjects (i.e., the Intention to treat population). Subjects will be analyzed according to their randomized treatment group. Analyses for demographics, baseline characteristics, and efficacy endpoints will utilize this analysis set. All summary
statistics of continuous variables will include: N, mean, median, standard deviation, minimum, maximum, and 95% confidence interval (except for safety laboratory assessment). All summaries presenting frequencies and incidences will include n, % and N, where N is the total number of subjects with recorded values in the corresponding arm. The comparisons of proportions (for dichotomous variables) between treatment group (Active vs. placebo) will be performed using logistic regression applying clinical center as a class variable (covariate) in the model. The comparisons of distribution location parameters (for means of continuous variables) between treatments groups will be compared based on the analysis of covariance (ANCOVA) models adjusting for stratification factors and baseline value.

The missing data for the dichotomous endpoints will be imputed using missing as “nonresponder method”. For continuous endpoints, the missing data will be imputed based on the ‘Baseline observation carried forward’ (BOCF) approach. Several sensitivity analyses will be applied to explore robustness of the findings - including “worst case”, “best case”, and multiple imputation techniques (details will be provided in ‘Statistical Analysis Plan’). Additionally, completer analyses will be performed on those who complete 6 months of treatment. During follow-up, any medical treatments which could potential modify the intestinal microbiome including antibiotics will be reported, but will not affect the statistical analysis. Statistical estimates will be presented as odds ratios (OR) with 95% Confidence Intervals (95% CI). Two-sided P-values for primary, secondary and exploratory endpoints will be computed and will not be adjusted for multiplicity. The P-values will be interpreted as nominal and descriptive.

Exploratory stratified analyses will investigate whether the treatment effect varies with the fecal microbiome analysis performed at follow-up compared to baseline (+/- long-term changes in the intestinal microbiome and intestinal inflammation). Patients receiving other types of DMARD’s, more than one intra-articular steroid injections, or biological treatments for PsA during follow-up will as non-responders represent the outcome group not fulfilling the primary outcome measure. Differences in demographics and baseline disease activity between this drop-out 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.

Adverse Events
Subject incidence rates of all treatment-emergent adverse events will be tabulated by system organ class and preferred term. Tables of fatal adverse events, serious adverse events, adverse events leading to withdrawal from investigational product or from study, and significant treatment-emergent adverse events, also will be provided. For the long term extension portion of this study, exposure adjusted event rates will be summarized.

PUBLICATIONS AND AUTHOR CONSIDERATIONS
In addition to being part of MK's PhD thesis, the aim of the project is to publish in English-language journals. The first publication will be an open access protocol publication. MK will be the first author and TE will be the last author. Data is expected to be published in minimum three papers, one with the primary and secondary outcome parameters; one with the tertiary parameters; and one regarding translocation of lactulose and mannitol through the mucosal barrier into the bloodstream.
All results, including positive, negative and in inconclusive results will be published.

The order of co-authors regarding primary and secondary outcome:

The order of co-authors regarding tertiary outcome:

The order of co-authors regarding translocation:

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<td>Study nurse salary</td>
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<td>Donor screening and compensation</td>
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<td>FMT procedure</td>
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<td>Blood tissue bank</td>
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<td>Intestinal translocation test</td>
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<td>Statistical assistance</td>
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<td>Total</td>
<td><strong>851,696 kr.</strong></td>
<td><strong>1,020,683 kr.</strong></td>
<td><strong>1,079,814 kr.</strong></td>
<td><strong>220,000 kr.</strong></td>
<td><strong>3,172,193 kr.</strong></td>
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DECLARATION OF INTERESTS
None of the team members of this research project has declared any potential conflict of interest.
APPENDICES

APPENDIX 1: PROTOKOLRESUMÉ
APPENDIX 2: DELTAGERINFORMATION (PATIENT)
APPENDIX 3: DELTAGERINFORMATION (DONOR)
APPENDIX 4: REKRUTTERINGSOPSLAG (DONOR)
APPENDIX 5: PATIENTVEJLEDNING SIGMOIDEOSKOPI
APPENDIX 6: SAMTYKKÆRKLÆRING (PATIENT)
APPENDIX 7: SAMTYKKÆRKLÆRING (DONOR)
APPENDIX 8: HEALTH ASSESSMENT QUESTIONNAIRE (HAQ)
APPENDIX 9: STUDY-COMPOSED QUESTIONNAIRE
APPENDIX 10A: CLINICAL DATA COLLECTION FORM (BASELINE)
APPENDIX 10B: CLINICAL DATA COLLECTION FORM (FOLLOW-UP)
APPENDIX 11: Spondylarthritis Research Consortium of Canada (SPARCC) enthesitis index
APPENDIX 12: PSORIASIS SEVERITY INDEX (PASI)
APPENDIX 13: MAYO ENDOSCOPIC SCORE
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[26]


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