(Transplantation of faeces in ulcerative colitis; restoring nature’s homeostasis)

TURN trial

(April 2013)
PROTOCOL TITLE: ‘Transplantation of faeces in ulcerative colitis; restoring nature’s homeostasis’

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<td>Transplantation of faeces in ulcerative colitis</td>
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<td>Date</td>
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<td>Laboratory sites &lt;if applicable&gt;</td>
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<td>Academic Medical Center</td>
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<td>Amsterdam, The Netherlands</td>
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## PROTOCOL SIGNATURE SHEET

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<td><strong>For non-commercial research, Head of Department:</strong></td>
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### LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

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<tr>
<td>ABR</td>
<td>ABR form (General Assessment and Registration form) is the application form that is required for submission to the accredited Ethics Committee (ABR = Algemene Beoordeling en Registratie)</td>
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<td>AE</td>
<td>Adverse Event</td>
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<td>AR</td>
<td>Adverse Reaction</td>
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<tr>
<td>CA</td>
<td>Competent Authority</td>
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<td>CCMO</td>
<td>Central Committee on Research Involving Human Subjects</td>
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<td>CV</td>
<td>Curriculum Vitae</td>
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<td>DSMB</td>
<td>Data Safety Monitoring Board</td>
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<td>EU</td>
<td>European Union</td>
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<td>EudraCT</td>
<td>European drug regulatory affairs Clinical Trials GCP Good Clinical Practice</td>
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<td>IB</td>
<td>Investigator’s Brochure</td>
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<td>IC</td>
<td>Informed Consent</td>
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<td>IMP</td>
<td>Investigational Medicinal Product</td>
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<td>IMPD</td>
<td>Investigational Medicinal Product Dossier</td>
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<tr>
<td>METC</td>
<td>Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)</td>
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<tr>
<td>(S)AE</td>
<td>Serious Adverse Event</td>
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<tr>
<td>SPC</td>
<td>Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)</td>
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<tr>
<td>Sponsor</td>
<td>The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.</td>
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<tr>
<td>SUSAR</td>
<td>Suspected Unexpected Serious Adverse Reaction</td>
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<td>Wbp</td>
<td>Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)</td>
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<td>WMO</td>
<td>Medical Research Involving Human Subjects Act (Wet Medisch-wetenschappelijk Onderzoek met Mensen)</td>
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SUMMARY

Rationale: Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) of the colon. Complaints such as abdominal pain, cramps and bloody diarrhoea usually start in early adulthood and lead to life-long substantial morbidity. Despite decades of research the etiology and pathogenesis of this disease are still poorly understood. Hence, there is no medical treatment available that meets the desired criteria of high efficacy versus low adverse effects. The current prevailing hypothesis regarding the cause of UC states that the pathogenesis involves an inappropriate and ongoing activation of the mucosal immune system driven by the intestinal microbiota in a genetically predisposed individual. Four non-mutually exclusive hypotheses have been proposed regarding the role of the microbiota in IBD: (i) a disbalance between protective and harmful bacteria (dysbiosis hypothesis); (ii) impaired intestinal barrier hypothesis; (iii) excessive immune response against normal microbiota; (iv) unidentified persistent pathogen hypothesis.

There are several clinical observations that support the dysbiosis hypothesis. (i) germ-free mice will not develop experimentally induced colitis; (ii) there are several papers on the beneficial effects of probiotics in mild to moderate colitis (iii) there are several case reports of patients achieving remission after faecal transplantation.

In an historic perspective, faecal transplantations or infusions have been given for over 50 years to patients with recurring Clostridium difficile infections. In these patients it has been shown that changes in intestinal flora contribute to the recurrent nature of Clostridium difficile infections in this particularly troubled group of patients. In literature there are over 200 patients described in case reports and case series who were cured from recurrent Clostridium infections.

Systematic investigation into the effect of correcting the dysbiosis in ulcerative colitis patients has never been performed. The most radical way to restore the presumably disturbed natural homeostasis in UC is to perform faecal transplantation from a healthy donor.

By designing a specific treatment protocol using faecal transplantation a unique opportunity is created to investigate the potential beneficial effects of restoring microbial homeostasis.

Objective: Primary objective: to study the effect of faecal transplantation in a phase II randomised placebo controlled design on simple clinical colitis activity index (SCCAI) and endoscopic Mayo score. Secondary objective: to study intra individual changes in microbiota composition of faeces and mucosal biopsies at t=0, t=6, and t=12 weeks after faecal transplantation.

Study design: This is a double-blind randomized placebo controlled clinical proof-of-concept study as well as a reversed translational part.
**Study population:** Patients with a mild to moderately active UC; defined as a simple colitis severity index (SCCAI) of ≥4, from the IBD centre of the AMC and patients from other hospitals will be enrolled. Donors: relatives or volunteers will serve as faeces donor, potential donors will be thoroughly screened.

**Intervention:** Patients will be treated with faecal transplantation, processed for duodenal-tube infusion. Faeces will be collected from a donor as well as the patient him/herself, in which their own faeces will be used as a placebo.

**Main study parameters/endpoints:**
- Complete clinical remission (SCCAI ≤2).
- Reduction of Mayo endoscopic inflammation score (decrement ≥1)
- Time to recurrence (recurrence is defined as a SCCAI of ≥4 and Mayo score ≥1)
- Adverse events (AE) at t=3, t=6 and t=12 weeks
- Frequency of bowel movements
- Intra individual changes in presence of microbial DNA in faecal samples at t=0, t=6, and t=12 weeks after faecal transplantation.
- Intra individual changes in presence of microbial DNA in mucosal biopsies at t=0, t=6, and t=12 weeks after faecal transplantation.

**Nature and extent of the burden and risks associated with participation, benefit and group relatedness:** It seems plausible that patients have benefit from donor faeces of strictly selected healthy donors. Sigmoidoscopies have a very small risk of complications; the same holds for cortrak duodenal tube positioning. According to our experience with human faecal transplantation in therapy resistant Clostridium difficile associated colitis as well as from the pilot trial studying the effects of faecal transplantation on obesity no serious side effects were observed.
1. INTRODUCTION AND RATIONALE

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) of the colon that affects approximately 20,000 individuals in The Netherlands. Complaints such as abdominal pain, cramps and bloody diarrhoea usually start in early adulthood and lead to life-long substantial morbidity. Despite decades of research the etiology and pathogenesis of this disease are still poorly understood. Hence, there is no medical treatment available that meets the desired criteria of high efficacy versus low adverse effects. Many patients will need at some point during their remitting disease course immunosuppressive medication, which is accompanied with potential serious side-effects. Even then, around 10% of patients experience serious flares refractory to intense medical treatment. In this situation, proctocolectomy with ileoanal pouch reconstruction is performed. The current prevailing hypothesis regarding the cause of UC states that the pathogenesis involves an inappropriate and ongoing activation of the mucosal immune system driven by the intestinal microbiota in a genetically predisposed individual. Four non-mutually exclusive hypotheses have been proposed regarding the role of the microbiota in IBD: (i) a dysbalance between protective and harmful bacteria (dysbiosis hypothesis); (ii) impaired intestinal barrier hypothesis; (iii) excessive immune response against normal microbiota; (iv) unidentified persistent pathogen hypothesis.

The human microbiota forms a highly complex ecosystem with its host, consisting of hundreds of different species of microorganisms, the majority of which have not yet been cultured. With the recent advent of small subunit rRNA (SSU rRNA) gene sequencing technology, it is now estimated that the cumulative number of specific gastrointestinal tract phylotypes is more than 1800, of which less than 25% can be identified with culture dependent approaches (1). Consequently, knowledge of the complex interplay between the microbiota and the innate and adaptive immune systems of its host is still in its infancy.

Recently, at the Laboratory of Microbiology of Wageningen University the so-called HITChip has been developed and extensively validated against FISH, qPCR, T-RFLP, as well as pyrosequencing for study of the human microbiota (2;3). This is a custom-made Agilent microarray targeting all currently known (approximately 1140 at present) gastrointestinal tract bacteria. It is a dynamic microarray in the sense that whenever new sequences are published these can instantly be added to the microarray. To date, the HITChip is the most comprehensive and up to date high throughput tool to study the complex composition of the microbiota.

There are several clinical observations that support the dysbiosis hypothesis. (i) germ-free mice will not develop experimentally induced colitis; (ii) there are several papers on the beneficial effects of probiotics in mild to moderate colitis (4-7); (iii) there are several case
reports of patients suffering from ulcerative colitis, irritable bowel syndrome, inflammatory bowel disease and constipation achieving remission after faecal transplantation (8).

In an historic perspective, faecal transplantations or infusions have been given for over 50 years to patients with recurring Clostridium difficile infections. In these patients it has been shown that changes in intestinal flora contribute to the recurrent nature of Clostridium difficile infections in this particularly troubled group of patients. In literature there are over 200 patients described in case reports and case series, who were cured from recurrent Clostiridum infections. No serious side effects were reported in this group of patients. Patients either received a faecal infusion from a healthy screened donor through an enema, duodenal or gastric infusion or through a colonoscopy.

In a randomised trial recently performed called: ‘the FECAL trial’ (Faecal therapy to Eliminate Clostridium difficile-Associated Longstanding diarrhoea), faecal infusions cured approximately 90% of patients dealing with recurrent Clostridium difficile infections.

The most radical way to restore the presumably disturbed natural homeostasis in ulcerative colitis patients is to perform faecal transplantation from a healthy donor. Systematic investigation into the effect of correcting the dysbiosis with faecal transplantation in UC has never been performed. However, formal investigation using a comparative approach has never been performed. In addition, some empirically administered faecal transplantations, mostly by enema, showed only a temporary effect on disease activity of not more than 3 months.

The reason for this phenomenon has not been investigated. Hence, although there are some reports of a highly favourable response the effect of faecal transplantation as well as the best way of administration is unknown.

Preliminary data of an ongoing double-blinded, randomised, controlled trial called: the FATLOSE (Fecal Administration To LOSE weight) in which healthy participants with metabolic syndrome were treated with donor faeces of healthy participants to investigate the effect on glucose homeostasis and (intestinal) inflammation, suggest that the composition of the microbiota of the recipient tends to revert back to its original composition between 2-6 weeks after single transplantation.

Moreover, due to the myriad of species and limitations of the so far applied techniques, the dysbiosis hypothesis is still an assumption. By designing a specific treatment protocol using faecal transplantation a unique opportunity is created to investigate the potential beneficial effects of restoring microbial homeostasis. If a substantial proportion of patients remains in clinical remission after one or two transplantations for at least one year, this would offer an interesting and cheap alternative to second or third line medical therapy regimens. In addition, comparing best to worst responders using the recently developed HITChip
microarray high-throughput technology will enable us to study in detail which component(s) is/are responsible for this equilibrium between the host and the human microbiota.
2. OBJECTIVES

We hypothesize that faecal transplantation from a healthy donor can restore the dysbiosis present in UC patients, thereby inducing remission of the chronic inflammation of the colonic mucosa.

The aim of our project is to perform a proof-of-concept study with regard to safety and efficacy of human faecal transplantation for induction of remission of colitis in patients with ulcerative colitis. In the same study we intend to investigate whether repeated transplantation is more efficacious than single transplantation. In addition, in a reversed translational fashion, we intend to study the changes in the microbiota in this unique experimental setting with the HITChip. With this novel high-throughput tool we expect to find important data regarding the disturbed interplay between the mucosa and particular constituents of the microbiota in UC.

Primary objective

• To study the effect of faecal transplantation in a phase II randomised placebo controlled design on simple clinical colitis activity index (SCCAI) (see appendix B(9)) and endoscopic Mayo score(10).

Secondary objective

• To study intra individual changes in microbiota composition of faeces and mucosal biopsies at t=0, t=6, and t=12 weeks after faecal transplantation.
3. STUDY DESIGN

This is a double-blind randomized placebo controlled clinical proof-of-concept phase IIa study with a subsequent reversed translational part. This study (treatment) will be done at the AMC, despite patients from other hospitals can be enrolled for the study too.

Time schedule:
01/04/2011: start recruitment.
End 2013: closure of enrollment, start HITChip microarray analysis.
Medio 2014: presentation of clinical and translational data

* donor faeces: processed as mentioned in ‘study procedures’
* placebo: recipients' own faeces processed as mentioned in ‘study procedures’
4. STUDY POPULATION

4.1 Population (base)

Patients
Patients with a mild to moderately active UC: defined as a simple colitis severity index (SCCAI) of ≥4, will be recruited from the IBD centre of the Department of Gastroenterology & Hepatology of the AMC. Currently, we care for approximately 1000 UC patients, from which we expect to recruit a small group of eligible patients. In addition, patients from other hospitals will be enrolled. Already, we receive numerous inquiries from UC patients throughout the country who have heard or read about faecal transplantation at our centre because of the FECAL trial and FATLOSE trial as described in the introduction of this protocol.

Donors
Healthy relatives or volunteers will be used as donors. Potential donors will be thoroughly screened as mentioned in ‘study procedures’.

4.2 Inclusion criteria

- Age ≥18
- Ability to give informed consent
- Established ulcerative colitis with known involvement of the left colon according to the Lennard-Jones criteria
- SCCAI of ≥4 and ≤11
- Endoscopic Mayo score of ≥1
- Stable dose of thiopurines in preceding 8 weeks
- Stable dose of corticosteroids and 5-ASA in preceding 2 weeks
- Women need to use reliable contraceptives during participation in the study

4.3 Exclusion criteria

Patients

- Condition leading to profound immunosuppression
  - For example: HIV, infectious diseases leading to immunosuppression, bone marrow malignancies
  - Use of systemic chemotherapy
• Anti-TNF treatment in preceding 2 months
• Ciclosporine treatment in preceding 4 weeks
• Use of Methotrexaat in preceding 2 months
• Prednisolone dose > 10 mg
• Life expectancy < 12 months
• Use of systemic antibiotics in preceding 6 weeks
• Use of probiotic treatment in preceding 6 weeks
• Positive stool cultures for common enteric pathogens (Salmonella, Shigella, Yersinia, Campylobacter, enteropathogenic e coli)
• Positive faecal PCR-test (positive PCR means: ≥ 1 of the following viruses is present) for: Rotavirus, Norovirus, Enterovirus, Parechovirus Sapovirus, Adenovirus 40/41/52. Astrovirus.
• History of surgery:
  o hemicolecotomie (defined as: surgery resulting in a resection of > ½ of the colon)
  o presence of a pouch due to surgery
  o presence of stoma
• Known intra-abdominal fistula
• Pregnancy or women who give breastfeeding
• Vasopressive medication, icu stay
• Signs of ileus, diminished passage
• Allergy to macrogol or substituents, eg peanuts, shellfish

Donors

• Abnormal bowel motions, abdominal complaints or symptoms indicative of irritable bowel syndrome
• Inflammatory bowel syndrome
• An extensive travel behaviour
• Occurent use of medication with potential side effects for the patient.
• Predisposing factors for potential transmittable diseases (e.g. regular sexual contact with prostitutes)
• Positive blood tests for the presence of: HIV, HTLV, Strongyloides.
• Active hepatitis A, B- or C-virus infection, acute infection with cytomegalovirus or Epstein-Barr virus
• Positive faecal tests for the presence of:
- **Bacteria:**
  C. difficile, Yersinia, Campylobacter, Shigella, Salmonella, enteropathogenic E. coli

- **Parasites:**
  - Dientamoeba histolytica, Giardia lamblia or Dientamoeba fragilis, Blastocystis hominis.
  - more than 1 of the following non-pathogenic parasites:
    Entamoeba gingivalis, Entamoeba hartmanni, Entamoeba coli, Entamoeba polecki, Endolimax nana, Iodamoeba bütschlii, m Entamoeba dispar, Entamoeba moshkovskii (If a donor turns out positive for only 1 of the above mentioned non-pathogenic parasites; inclusion is acceptable)

- **Viruses** (faecal PCR-test):
  for: Rotavirus, Norovirus, Enterovirus, Parechovirus Sapovirus, Adenovirus 40/41/52.Astrovirus. (positive PCR means: ≥ 1 of the viruses is present)

- Risk of Creutzfeldt Jacob’s disease
- History or current use of iv drugs
- History of treatment with growth factors
- Untreated infection with: Treponematoses, TBC, Herpes virus
- Antibiotic treatment in the past 8 weeks

### 4.4 Sample size calculation

Since this is a proof-of-concept study a reliable sample size calculation is not feasible. Borody et al. (11) reported a longstanding medication-free remission in 6 patients following single faecal transplantation by enema. In their entire series of 40 patients they observed a remission following single enema in > 70%. However most of them relapsed within one year (personal communication). Most probiotica studies show a placebo effect of 15-30 % at t=12 weeks. Based on these figures and predefining a desired power of 80% and a two-sided $\alpha$-level of 0.05, a total sample size of N=42 would suffice. Alternatively, assuming an average improvement in Mayo score of 0 in the placebo group and 1.5 in the treated group would require a total group size of N=27. Based on accumulated evidence with HITChip analysis, a sample size of 20 individuals per group is normally enough to detect relevant differences in the microbiota especially with pair-wise comparison. Hence, a total sample size of N=40 seems adequate.

Sample size assumption
Interim analyse on safety and efficacy will be performed by the DSMB. If the trial turns out to be effective, but the treatment effect seems less then 70% mentioned in the calculation in this paragraph, the DSMB is allowed to purpose an assumption or recalculation in sample size.

Due to costs and practical reasons the treatment group has a maximum of 80 patients.
5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment
Patients will get bowel lavage at home, this constitutes of 4 liters of macrogol electrolytes solution. After that they will be treated with faecal transplantation, processed for tube infusion.

Faeces will be collected from a donor as well as the patient him/herself, in which their own faeces will be used as a placebo. Both placebo as well as donor faeces will be processed according to the same protocol.

Quality of faecal infusion:

Screening of the donor:
Potential donors will be thoroughly screened on possible infectious diseases. After screening we will keep close contact to the donor, and just before faecal transplantation donors will be asked again about potential risks of infectious diseases in the window phase.

Time from production to infusion
In several reports on faecal infusion in patients with diarrhea due to a Clostridium difficile infection it is stated that faeces are processed and infused as quickly as possible following production by the donor, in order to preserve faecal flora. Due to lack of detailed data it is not possible to establish a relationship between a prolonged time that has passed between production and infusion, and failure of therapy.

In our department of microbiology faecal samples from a healthy donor processed for tube infusion were cultured 3 and 6 hours after production by the donor. Large amounts of aerobic and anaerobic bacteria were cultured. Unfortunately the results were not published. But infusion until six hours after production by the donor seems safe.

Microbiota present
Although there can be potential important differences in the quality of the microbiota present in donor faeces from different individuals, historically their intestinal flora has not been analysed prior to use for faecal infusion. Information is lacking with regard to the specific groups and amount of bacteria necessary for optimal restoration of intestinal flora.

A possible complication of faecal infusion could be bacterial overgrowth in the small intestine after infusion, this complication did not occur in the FECAL trial patient group.

Processing of the product, hygiene and storage of faecal product
Faeces will be processed by an experienced research nurse. Processing will be done according to a strict protocol. This will be done at the department of microbiology in a very clean environment. For mixing of the product only disposable material will be used. Faeces will be put into a closed glass bottle and will not be unattended until infusion.

5.2 Use of co-intervention
Not applicable

5.3 Escape medication
In case of nausea or vomiting anti-emetics can be used, iv or oral.
6. METHODS

6.1 Study parameters

6.1.1 Main study parameters

• Co-primary endpoint of clinical remission (SCCAI ≤2; (10)), as well as reduction of Mayo endoscopic inflammation score (decrement ≥1) as assessed by sigmoidoscopy prior to and at 12 weeks after treatment.

6.1.2 Secondary study parameters

• Adverse events (AE) at t=3, t=6 and t=12 weeks
• SCCAI score reduction at t=6 weeks (pairwise SCCAI reduction of > 1.5 points will be regarded as a relevant reduction (10))
• Frequency of bowel movements (starting to report at t= -2 weeks)
• Reduction of Mayo endoscopic score at t=6 wk
• Time to recurrence (recurrence is defined as a SCCAI of ≥4 and Mayo score ≥1 (10))
• Intra individual changes in presence of microbial DNA in faecal samples at t=0, t=6, and t=12 weeks after faecal transplantation.
• Intra individual changes in presence of microbial DNA in mucosal biopsies at t=0, t=6, and t=12 weeks after faecal transplantation.

6.1.3 Other study parameters

• SCCAI at 4, 6, 8, 10, and 12 months
• AE assessment 4, 6, 8, 10, and 12 months
• Use of medication during the study period

6.2 Randomisation, blinding and treatment allocation

Randomization will be performed after the baseline sigmoidoscopy when patient meets all eligibility criteria. Mucosal biopsy samples will be taken during the baseline endoscopy. Alea software will be used to perform randomisation. Patients will be randomised to one of two treatment arms: two times treatment with placebo or two times treatment with donor faeces. Blinding will be guaranteed by collecting donor as well as recipients’ faeces (which is used as a placebo). On both days of faecal transplantation both patient and donor will deliver faeces produced that morning. Randomisation and preparation of the faeces will be performed by one of the research nurses, she is the only person who will know which treatment the patient
will be given and will have no role in further part of the study. In case of any question about preparation of faeces, an independent physician with experience in faecal infusion can be consulted. Faeces will be put in a 500 ml glass bottle and will look like a brownish fluid not recognizable as faeces from the donor or patient and given to the investigator who will perform nasoduodenal tube infusion. On the second day of transplantation again both donor as well as patients’ faeces will be delivered, and according to the randomisation performed on the first day, faeces will be processed by the research nurse and given to the investigator. Both patient, donor, endoscopist, nurses and investigator will be blinded. Ample experience with this procedure has been obtained in the FATLOSE protocol.

Since treatment can be given aside of medicinal treatment, there’s no indication for breaking the randomisation code. De-blinding of investigators may take place if the total sample size is reached and all patients completed their primary endpoint (t:12 wks), in order to publish the data. Patients will be blinded until Follow-up data until the end of the study (t:12 months) is collected.

6.3 Study procedures
A similar study ‘the FECAL trial’ (12;13) was performed in patients with recurrent Clostridium difficile in our hospital. Therefore, study procedure according to screening of donors, preparation of faeces as well as procedure of transplantation is based on this trial; in addition, screening for pathogenic gastrointestinal viruses will be performed.

Screening
Donors
See for screening and study visits schedule appendix A.
Potential donors will be thoroughly screened according to the protocol used in the FECAL trial as previously approved by our METC.
Prior to screening of faeces and blood, potential donors have to fill in an extensive questionnaire (see ‘F1B1_vragenlijst_donoren’ attached with this protocol). They are asked for their vaccination status regarding Hepatitis A and B. Donors with abnormal bowel motions, abdominal complaints, or symptoms indicative of irritable bowel syndrome, an extensive travel history or predisposing factors for potential transmittable diseases are excluded. Donors are not allowed to take any medicine with potential side effects for patients. If donors are considered eligible after completing the questionnaire, they are screened as mentioned:
All donors will be examined for the presence of HIV, HTLV, hepatitis B- and C-virus, and acute infection with cytomegalovirus or Epstein-Barr virus. Furthermore hepatitis A will be tested if not vaccinated. Faeces will be examined for the presence of C. difficile, Yersinia, Campylobacter, Shigella, Salmonella, enteropathogenic E. coli and parasites. Specific tests will be performed for the presence of Giardia lamblia, Dientamoeba fragilis and Dientamoeba histolytica.

In addition, a multiplex PCR containing probes against enteral viruses (Rotavirus, Norovirus, enterovirus parechovirus, sapovirus, adenovirus 40/41/52, astrovirus) will be done on faeces. All viruses described above are agents that can cause gastroenteritis or can be found in asymptomatic carriers.

Viruses may persist 2 days to 6 weeks after clinical signs of gastroenteritis. Rotavirus, Norovirus, Enterovirus and Parechovirus, are described or suspected in asymptomatic carriers and might cause problems in recipients.

If a donor turns out eligible for faecal donation after screening, he or she is allowed to donate faeces in the next 3 months after screening. If this period is expired, the complete screening will be repeated.

Additional screening if donors have signs of, or are at risk for an infection.

Donors need to complete: donor questionnaire: ‘F1B2_vragenlijst_donoren II’ before every faecal donation.

- If there is, during the eligible ‘donation period’, a suspicion of a viral enteric infection (symptoms of gastroenteritis, fever, a sore throat, thickened lymph nodes): multiplex PCR on faeces will be performed 3 weeks after the symptoms have passed.
  - In case of a positive test result: the donor will be excluded
  - In case of a negative test result: the donor is allowed to donate faeces from that moment.

- If there is, during the eligible ‘donation period’, a suspicion or risk for other infections (bacterial gastroenteritis/ risk for HIV/ STD etc) according to the questionnaire: Donors will be excluded or additional faecal or serologic tests will be performed on indication.

**Patients**

See for screening and study visits schedule appendix A.

When patients are eligible according to the inclusion criteria and SCCAI index they will be asked to give informed consent. After that patients will be screened for EBV, CMV and HIV. Faeces will be tested on common enteric pathogens by:
Triple faeces test; Dientamoeba histolytica, Giardia lamblia, Dientamoeba fragilis
Culture/ toxin testing; Salmonella, Shigella, Yersinia, Campylobacter, Clostridium difficile, enteropathogenic E coli
Multiplex PCR; Rotavirus, Norovirus, enterovirus, parechovirus, sapovirus, adenovirus 40/41/52, astrovirus.

A sigmoidoscopy will be performed. If a left sided ulcerative colitis with involvement of the left colon according to the Lennard-Jones criteria is established and endoscopic Mayo score is \( \geq 1 \), baseline mucosal biopsy samples will be taken.

All eligible patients with mild to moderately active UC, will be randomised to one of two different treatment arms (figure 1). A \( (n=20) \): faecal transplantation at t=0 and t=3 weeks; B \( (n=20) \): placebo at t=0 and t=3 weeks.

Patients will be pre-treated with a bowel lavage with 2 liters of macrogol electrolytes (Moviprep ®) plus two liters of clear fluids at home, the day prior to the transplantation.

**Preparation of faeces**
Donor or recipients’ own faeces will be prepared using normal saline as mentioned in fig 2. A minimum of 60 g of faeces will be dissolved and after preparation a suspension of +/- 500 ml is created. Faeces are installed within six hours after production.

**Amsterdam protocol used for the preparation of donor faeces**

1. Faeces are collected and weighed (ca. 60-120 g, depending on production);
2. 300–600 ml 3% saline (0.9% NaCl) is added and mixed until a smooth suspension is created;
3. Faeces are poured through a double gauze and put in a glass bottle;
4. Within six hours after production by the donor, the faeces are installed through a nasojunal tube.

Fig 2 preparation of donor faeces (13)

**Faecal transplantation procedure**
Patients will receive faecal infusion through a nasoduodenal tube. The tube is placed with use of a cortrack device, or endoscopically.
After that the suspension will be infused into the jejunum via a duodenal catheter.

**Assessments**
Patients will be followed by telephone in the weeks and months scheduled for assessments.
SCCAI will be assessed at t=0, 6 and 12 wks, 4, 6, 8, 10, and 12 months.
AE forms will be assessed at t=3, 6 and 12 weeks, 4, 6, 8, 10, and 12 months.
Stool frequency assessment starting at t=-2 weeks.

'Double check’ on primary endpoint data.
Data on both contents of the primary and secondary endpoint; endoscopy inflammation score (Mayo score) & clinical score (SCCAI) will be revised in order to ensure data quality.

- Confirmation of Mayo scores
  A Critical Event Commitee (CEC) consisting of: two IBD-specialised Gastroenterologists from the AMC (Principle investigator Dr. C. Ponsioen & prof G. D’Haens) will revise all endoscopy pictures.
  - the CEC will be blinded for: patient name/ information/ date and time point related to the endoscopy pictures.
  - This commitee may overrule the original endoscopy result.
  - In case of dis-agreement about the endoscopy score a third party will be invited to discuss the picture
  - All Mayo scores will be revised before un-blinding.

- clinical score (SCCAI)
  All SCCAI scores will be re-scored by a medical student and entered in a copy database. Differences in data will be checked by the investigator.

Collecting patients’ material
Sigmoidoscopy with mucosal biopsy samples will be performed at t=0, 6 and 12 wks, and when recurrence is suspected on the basis of SCCAI≥4.
(Faecal samples will be collected prior to, at t=6 wks, and 12 wks.)

Tissue handling and analyses on microbiota
Mucosal biopsies as well as faecal specimens (donor only faecal specimen) will be snap frozen as well as fixed in Carnoy solution and subsequently embedded in paraffin in order to preserve the mucus layer and adherent mucosal bacteria. Since the bacterial diversity in these samples is unknown, samples will be screened for diversity by Denaturing Gradient Gel Electrophoresis of 16S rRNA genes first. Part of the tissue material will be homogenised and subsequently analysed with the HITChip. This is a custom-made Agilent microarray recently designed by the Laboratory of Microbiology at Wageningen University(3), which contains more than 5000 oligonucleotide probes targeting all currently known (approximately 1140) gastrointestinal tract bacteria. The HITChip enables simultaneous SSU rRNA-based phylogenetic fingerprinting and relative quantification. Its high-throughput character makes it
especially suitable for our protocol, because of the high number of samples (approximately 540). Since we assume that the dysbiosis hypothesis is located at the mucosa adherent microbiota level, primary analysis will be done on the mucosa biopsy samples. Later on, both faecal samples of the donor as well as 3 faecal samples of the patient will be analysed by HITChip to compare distribution of microbial DNA of the donor faeces to the distribution in faeces of the patient.

Samples will be pairwise analyzed with the HITChip (at t=0 and t=6 wks and 12 wks within individual patients) to avoid the potential caveat of inter-individual variation, which can be substantial and is one of the reasons so much equivocal results have been published so far. Subsequently, depending on the results, immunological measurements will be performed on the biopsies to address the relation between the altered microbiota and the innate immune defense.

6.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

6.4.1 Specific criteria for withdrawal

Not applicable.

6.5 Replacement of individual subjects after withdrawal

Withdrawn patients will be replaced during the recruitment period in order to attain the desired sample size.

For further explanation of implementation -> pag 30. Fig 1. Additional randomisation in different situations.

6.6 Follow-up of subjects withdrawn from treatment

Withdrawn patients will be followed for SAE’s until 12 months after inclusion.
6.7 Premature termination of the study

Premature termination of the study will occur on patients’ request or when a patient is lost to follow-up.
7. SAFETY REPORTING

7.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects’ health. The investigator will take care that all subjects are kept informed.

7.2 Adverse and serious adverse events

Subjects are submitted to 3 sigmoidoscopies with tissue sampling for this study. In every case colonic biopsies are accompanied by a minimal risk of perforation, bleeding and infection. These complications are cited in the written patient information. Because strict conditions are there for donors, the risk of spreading potential pathogens during faecal transplantation seems nil.

Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational treatment. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

A serious adverse event is any untoward medical occurrence or effect that results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients’ hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, lack of efficacy of an IMP used for the treatment of a life threatening disease, major safety finding from a newly completed animal study, etc.

All SAEs associated with the given treatment will be reported within 24 hours to the members of the DSMC as mentioned in DSMB charter (attached with this protocol). After evaluation, members of the DSMB will decide if the accredited METC that approved the
protocol needs to be informed. Advice of the DSMB according to continuation or stop of the study will be reported to the METC.

SAEs that result in death or are life threatening will be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

7.2.1 Suspected unexpected serious adverse reactions (SUSAR)

Possible adverse reactions after faecal transplantation in this patient group:

- Temporarily increase of symptoms of diarrhea. In the patient group of the FECAL trial, in which patients with recurrent clostridium infection were given faecal transplantation, increase of bowel movements occurred for one or two days but the frequency of bowel movements returned to earlier habits or improved to a normal frequency of stool production. We assume that patients with recurrent clostridium infections have similar mucosal inflammation to our patient group with ulcerative colitis.

- Very small chance of bacterial translocation due to faecal transplantation. This adverse event did occur neither in the patient group of the Fecal trial nor FATLOSE trial.

- Psychological effect of faecal transplantation: we are very aware that faecal transplantation might be a psychological burden for the patients. Patients will be informed extensively and the procedure will be performed in the pace the patient can handle.

- Nasoduodenal tube position with cortrak: in this procedure there is a risk of perforation or positioning of the tube not in the duodenum as attempted but in the stomach. If there is any doubt of malposition of the tube, gastroscopy will be performed to place the tube correctly and faecal transplantation will be postponed until correct position is achieved.

- Vomiting due to the infusion of faeces. In case of nausea or vomiting anti-emetics can be used. In case of persisting vomiting faeces will be infused by colonoscopy.

- Very small risk of transmission of infectious diseases in the so called ‘window phase’, due to extensive screening the risk will be minimized.
The sponsor will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study investigational product, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

- The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

### 7.2.2 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, Medicine Evaluation Board and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.
7.3  **Follow-up of adverse events**

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

7.4  **Data Safety Monitoring Board (DSMB)**

In order to optimize safety of the study during inclusion patient data will be disclosed to a data safety monitoring board when 50 % of the intended sample size is attained and has reached 12 weeks follow up. The DSMB will consist of two independent members who will have no conflict of interest with the study. Members of the Data safety monitoring Committee will be: prof J. Tijssen (department of Clinical epidemiology and statistics) en dr. J. van der Meer (department of Internal medicine AMC).

A DSMB-charter is attached to this protocol.

The advice(s) of the DSMB will be notified upon receipt by the sponsor to the METC that approved the protocol. With this notification a statement will be included indicating whether the advice will be followed.
8. STATISTICAL ANALYSIS

8.1 Descriptive statistics

Primary analyses:

In this ‘intention to treat’ design, data on all randomised patients will be analysed, with exception of data of patients described in situation 1A. (fig. 1)

‘ Replacement of subjects’ / additional randomisation will be performed according to the following schedule:

Fig 1. Data collection & additional randomisation in different situations.

<table>
<thead>
<tr>
<th>Situation</th>
<th>Timepoint study</th>
<th>Sec endpoint (t: 6 wks) (endoscopy + SCCAI)</th>
<th>prim endpoint (t: 12 wks) (endoscopy + SCCAI)</th>
<th>Action on total sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A use of antibiotics (effect on success rate therapy)</td>
<td>t: &lt; 6 weeks</td>
<td>not evaluable</td>
<td>not evaluable</td>
<td>Randomisation: + 1 extra</td>
</tr>
<tr>
<td>1B use of antibiotics</td>
<td>t &gt; 6 weeks</td>
<td>evaluable</td>
<td>Last Observation Carried Forward (LOCF): 12 wks data = 6 wks data</td>
<td>-</td>
</tr>
<tr>
<td>2A start additional medicinal treatment because of ‘ongoing symptoms’</td>
<td>t: &lt; 6 weeks</td>
<td>not evaluable Score as: 0 = therapy failure</td>
<td>not evaluable Score as: 0 = th failure NRI</td>
<td>Randomisation: + 1 extra</td>
</tr>
<tr>
<td>2B start additional medicinal treatment because of ‘ongoing symptoms’</td>
<td>t &gt; 6 weeks</td>
<td>evaluable</td>
<td>not evaluable Score as: 0 = th failure LOCF</td>
<td>-</td>
</tr>
<tr>
<td>3A study termination: reason not disease related</td>
<td>t: &lt; 6 weeks</td>
<td>Score as: 0 = therapy failure (with exception if SCCAI score ≤ 2 at the moment of study termination)</td>
<td>12 wks data = 6 wks data NRI</td>
<td>Randomisation: + 1 extra</td>
</tr>
<tr>
<td>3B study termination: reason not disease related</td>
<td>t &gt; 6 weeks</td>
<td>evaluable</td>
<td>12 wks data = 6 wks data Last observation carried forward</td>
<td>-</td>
</tr>
</tbody>
</table>

Secondary analyses
A (sub-) analyses will be performed on data of all patients who completed both treatments; data on patients described in situation 1A, 2A, 3A in the schedule, will not be used for sub-analyses.

Clinical and endoscopic data will be pairwise compared using cross-tabulation and Wilcoxon testing.

HITChip data will be extracted from microarray images using the Agilent Feature Extraction Software and will be analysed in a custom designed relational database which runs under the MySQL database management system (http://www.mysql.com) using a series of custom made R scripts (1)(3). Cluster analysis and similarity of the microbiota profiles, expressed as Pearson correlation, will be assessed.

**Multivariate analysis**

In addition, multivariate analysis of the microbiota composition (identifying discriminating bacterial profiles) will be performed using CANOCO for Windows v4.5. This will allow determining the impact and significance of several variables (such as health status, gender and GI location) on the variation observed in the HITChip data.

**Interim analysis**

A DSMB is established to perform ongoing safety surveillance and to perform interim analyses on the safety data. Interim analyses will be done halfway the study, when every study arm has attained 10 patients who completed both treatments, and reached 12 weeks follow-up.

Clinical data will be compared using cross-tabulation and Wilcoxon testing.

- Stopping rules will be: (i) unacceptable occurrence of SAEs as judged by the DSMB members in conjunction with the METC at the AMC.

**Handling of protocol violations**

Change in immunosuppressive medication, including mesalazine derivatives, as well as antibiotics and probiotics are not allowed during the first 12 weeks of the study.

See for implementation Fig 1. Data collection & additional randomisation in different situations.
9. ETHICAL CONSIDERATION

9.1 Regulation statement
The study will be conducted according to the principles of the Declaration of Helsinki (version October 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

9.2 Recruitment and consent

UC patients
Patients with active UC, who fulfils the eligibility criteria, will be asked to participate by his/her treating physician at the outpatient clinic of the department of Gastroenterology and Hepatology of the Academic Medical Center, University of Amsterdam. Patients from other hospitals who have heard about faecal transplantation or read about this trial will be enrolled too.
A written (patient) information letter as well as an informed consent form will be provided to the patient and he/she will be allowed two weeks to consider his/her decision to participate.

Donors
Patients can ask their relatives or family members to participate. In case of volunteers, students or healthy employees from the Amc will be recruited by advertisements (attached with this protocol).
Potential donors will get a written (donor) information letter and will be given two weeks to decide whether or not to participate. After signing informed consent screening will start.

9.3 Benefits and risks assessment, group relatedness
Despite decades of research the aetiology and pathogenesis of ulcerative colitis is poorly understood. The hypothesis regarding to the role of microbiota in IBD as mentioned before, it seems plausible that patients have benefit from donor faeces of strictly selected healthy donors.
The risks associated with participation can be considered negligible because of minimal invasive treatment. Sigmoidoscopies have a little risk of complications, and accordance to experience with human faecal transplantation in therapy resistant Clostridium difficile associated colitis as well as the pilot studying the effects of faecal transplantation on obesity no serious side effects were reported.
9.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450,000.-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3,500,000.-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5,000,000.-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

9.5 Incentives

Donors will get a small present after (every) donation of faeces if they participate in the study, which will be a voucher of 10 euro’s. Also travel costs will be compensated.

For patients travel costs will be compensated.
10. ADMINISTRATIVE ASPECTS AND PUBLICATION

10.1 Handling and storage of data and documents
Patient data are filed encrypted on site and stored directly on a server hosted by the Intranet of the Academic Medical Centre, Amsterdam. This server is accessible by 2 medical doctors. The key to the code is safeguarded by the principal investigator. In addition, members of the Data safety Monitoring Committee will get permission to patient data on regard to safety. Because this study will be performed double blind, 2 people from the monitoring department at the Academical Medical Centre will get permission to have access in the database. During the study surveillance on safety, futility or positive efficacy can be done. Patient material will be safely stored at the Tytgat institute for liver and intestinal research, and transported to the Laboratory of Microbiology, Wageningen University for HIT-chip analyses. Specimens will be stored in Wageningen until 10 years after finishing the study.

10.2 Amendments
Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

10.3 Annual progress report
The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

10.4 End of study report
The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient’s last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final
study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

10.5 Public disclosure and publication policy
Public disclosure and publication of the research data are at the discretion of the investigators.
REFERENCES


Appendix A
Study parameters/ procedures TURN trial

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<th>( t = 6 )</th>
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## Appendix B

SCCAI index

**Clinical scoring system for the Simple Clinical Colitis Activity Index**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
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<td>1–3</td>
<td>0</td>
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<tr>
<td>4–6</td>
<td>1</td>
</tr>
<tr>
<td>7–9</td>
<td>2</td>
</tr>
<tr>
<td>&gt;9</td>
<td>3</td>
</tr>
<tr>
<td>Bowel frequency (night)</td>
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</tr>
<tr>
<td>1–3</td>
<td>1</td>
</tr>
<tr>
<td>4–6</td>
<td>2</td>
</tr>
<tr>
<td>Urgency of defecation</td>
<td></td>
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<tr>
<td>Hurry</td>
<td>1</td>
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<tr>
<td>Immediately</td>
<td>2</td>
</tr>
<tr>
<td>Incontinence</td>
<td>3</td>
</tr>
<tr>
<td>Blood in stool</td>
<td></td>
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<tr>
<td>Trace</td>
<td>1</td>
</tr>
<tr>
<td>Occasionally frank</td>
<td>2</td>
</tr>
<tr>
<td>Usually frank</td>
<td>3</td>
</tr>
<tr>
<td>General well being</td>
<td></td>
</tr>
<tr>
<td>Very well</td>
<td>0</td>
</tr>
<tr>
<td>Slightly below par</td>
<td>1</td>
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<tr>
<td>Poor</td>
<td>2</td>
</tr>
<tr>
<td>Very poor</td>
<td>3</td>
</tr>
<tr>
<td>Terrible</td>
<td>4</td>
</tr>
<tr>
<td>Extracolonic features</td>
<td>1 per manifestation</td>
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</tbody>
</table>

Appendix C
Mayo score

Mayo Endoscopic Scoring of Ulcerative Colitis

- Mayo UC Endoscopic Score = 0
  (Normal or inactive disease)

- Mayo UC Endoscopic Score = 1
  (Mild disease: erythema, decreased vascular pattern, mild friability)

- Mayo UC Endoscopic Score = 2
  (Moderate disease: marked erythema, abnormal vascular pattern, friability, erosion)

- Mayo UC Endoscopic Score = 3
  (Severe disease: spontaneous bleeding, ulceration)