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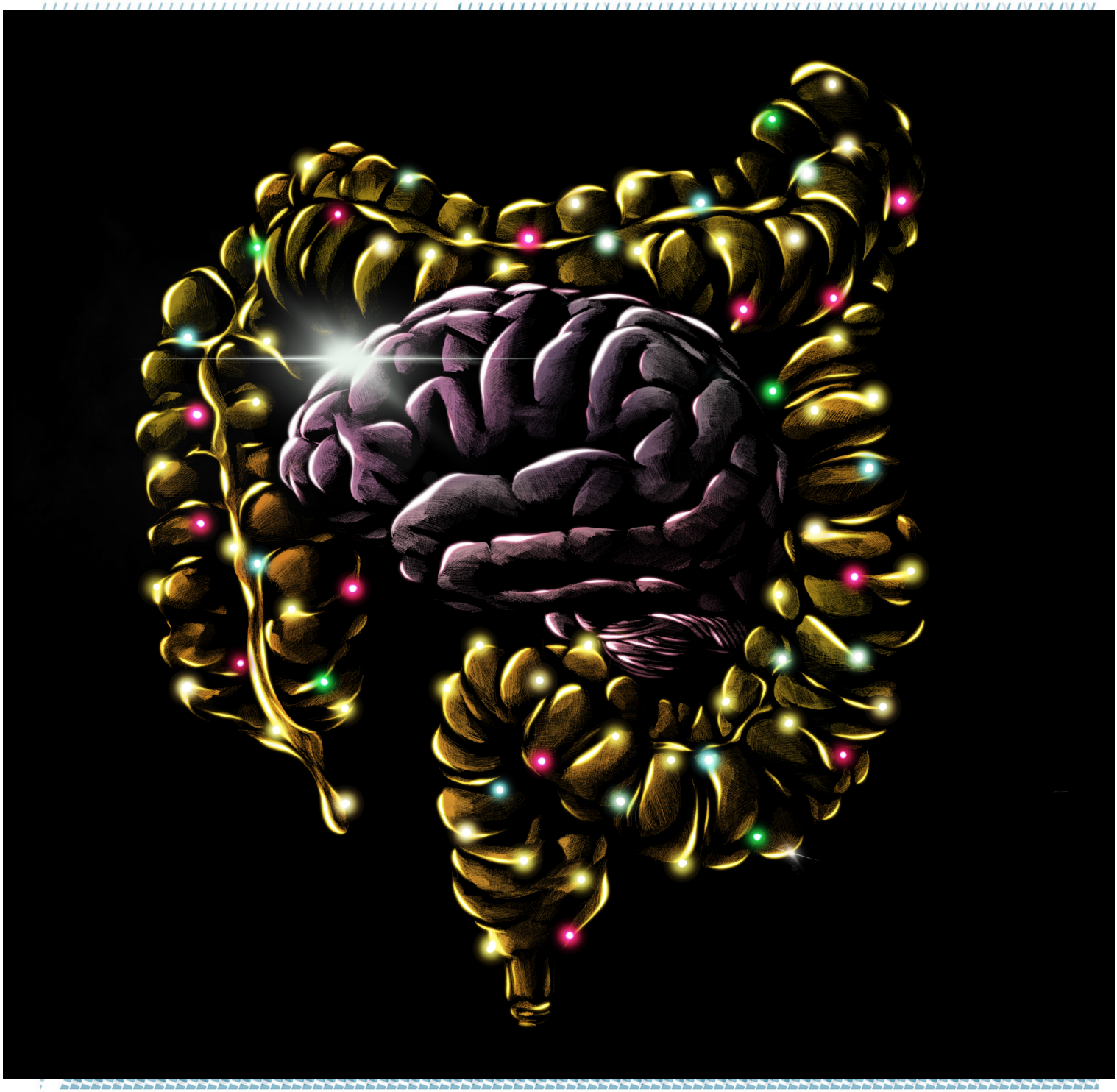
THE EFFECT OF FAECAL MICROBIOTA TRANSPLANTATION IN IRRITABLE BOWEL SYNDROME

A double blind, randomized placebo controlled single centre study

Peter Holger Johnsen
A dissertation for the degree of Philosophiae Doctor January 2020



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By

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"It's been a hard days night, and I've been working like a dog"

The Beatles

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Abstract

Irritable bowel syndrome (IBS) is common disorder of the lower gastro intestinal tract associated with a poor quality of life. Revealing the interplay between the microbiota and the host has lead to a better understanding of IBS. A disturbance in the bidirectional communication in the microbiota-gut-brain axis is suggested to be involved in the pathophysiology of IBS. The microbiota in this context is the bio-ecological community composed of multiple microorganisms in the gut. We hypothesised relief of symptoms in IBS from colonic infusion of faecal suspension from healthy donors, referred to as faecal microbiota transplantation (FMT).

In a double blind, randomized, placebo controlled, single centre trial we compared the effect of donor vs autologous FMT, delivered by colonoscopy, in patients diagnosed by the Rome 3 criteria. We also assessed the prevalence of differential diagnoses to the diagnosed participants.

We included 90 participants. Three participants did not show up for treatment and four were diagnosed with microscopic colitis by pinch biopsies obtained during the treatment procedure. Thus, 83 of the 90 included remained in a modified intention-to-treat analysis. The primary endpoint three months after treatment, defined as the proportion of responders with relief in gastrointestinal complaints, showed 65% responders in the donor FMT group vs 43% in the autologous FMT group (P=0,049). We found a corresponding improvement in fatigue and quality of life six months after treatment. These findings support initiating a phase three multi centre study to evaluate implementation of FMT as treatment for IBS in clinical practice.

The secondary analysis assessing the timeline of the FMT effect suggested a profound and sustainable treatment response in subgroups of participants. The results support the concept of the involvement of the microbiota-gut-brain axis in IBS pathophysiology, as gastro intestinal complaints, poor quality of life and fatigue were available for FMT therapy in subgroups of participants. The lack of effect in certain subgroups could be explained by a dominating central disturbance not affected by FMT therapy.

-List of papers

Paper 1

Hilpüsch F, Johnsen PH, Goll R, Valle PC, Sørbye SW, Abelsen B. Microscopic colitis: a missed diagnosis among patients with moderate to severe irritable bowel syndrome. Scand J Gastroenterol 2016;1–5

Paper 2

Johnsen PH, Hilpüsch F, Cavanagh JP, et al. Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial. Lancet Gastroenterol Hepatol 2018;3

Paper 3

Johnsen Peter Holger, Hilpüsch Frank, Valle Per Christian GR. The effect of faecal microbiota transplantation on IBS related quality of life and fatigue in moderate to severe non-constipated irritable bowel: Secondary endpoints of a double blind, randomized, placebo-controlled trial. Published online EBioMedicine 2019 Dec: 51:102562

Abbreviations

IBS = irritable bowel syndrome

FMT = faecal microbiota transplantation

IBS-D/M/C = IBS-Diarrhoea/Mixed/Constipated

FODMAP = Fermentable oligo-, di-, mono-saccharide and polyol

IBS-QoL = Irritable Bowel Syndrome Quality of Life

IBS-SSS = Irritable Bowel Symptom Severity Score

FIS = Fatigue Impact Scale

RM-ANOVA = Repeated Measures ANalysis Of VAriance

EMA = The European Medicines Agency

FDA = Food and Drugs Administration (USA)

1 Introduction

Irritable bowel syndrome (IBS) is a functional gut disorder, which by the Rome 3 criteria is characterized by abdominal pain or discomfort associated with abnormal frequency and consistency of bowel movements[1]. The current Rome 4 criteria focus on abdominal pain to avoid the poorly defined term ‘’discomfort’’[2]. IBS presents in a continuum between three phenotypes; IBS with diarrhoea (IBS-D), IBS with constipation (IBS-C) and IBS with both diarrhoea and constipation (IBS-M). Traditionally, IBS pathophysiology is explained as a disturbance in gastro-intestinal motility, visceral sensation, and brain-gut interaction where psychosocial distress predisposes to, and exaggerates the disease burden. However, IBS is a heterogeneous disorder, and there is now emerging evidence for distinct pathophysiological subtypes, which extends beyond the traditional phenotypic subtyping of IBS based on stool frequency and consistency[3–5]. The most prevailing hypothesis is that IBS is a mixed disorder involving elements of the gut-brain axis, diet, genetic factors, infections and disturbances in the intestinal microbiota, low-grade mucosal inflammation, immune activation, altered intestinal permeability, disturbed bile salt metabolism, abnormalities in serotonin metabolism and alterations in brain function[4–6]. A better understanding of these mechanisms may provide options for more targeted and individualized therapies.

1.1 Epidemiology of irritable bowel syndrome

The epidemiologic characteristics of IBS remain uncertain because of heterogeneity in studies. In addition to using different diagnostic criteria the survey methodology is not consistent. The pooled global prevalence of IBS is estimated to 11,2% but remains elusive with considerable regional differences. For a more detailed review see[7,8]. Prevalence peak at ages 25-35 in women and 30-50 years in men[9]. A significant difference in prevalence in individual countries is found (1,1% in France and Iran to 35,5% in Mexico)[7]. A public health survey from Norway found IBS (Rome 2 criterion) in 8% of 4622 subjects that completed the survey[10]. IBS subtypes differ depending on the study population and diagnostic criteria applied, but overall the distribution seems even[11–13].

The occurrence of IBS in women are approximately 1.5 – 3-fold higher than seen in men, and occurs in all age groups for both genders[11,14] One North American Study found the incidence rate of IBS two per 1000 per year. The true incidence is probably higher, as many IBS

patients are not diagnosed[15]. In a study pulation of patients with a previous history of gastro-intestinal complaints 43,1% was undiagnosed IBS[16].

1.2 Diagnosing IBS

The diagnostic criteria for IBS have been under five revisions from the Manning Criteria in 1978 and until now by the last iteration of the Rome criteria (Rome 4) in 2016[17,18]. Table 1 shows an overview of the revised diagnostic criteria from past until present. At the time we started our study, Rome 3 was the current iteration with a sensitivity of 68.8% and specificity of 79.5% in a secondary care patient population[1]. Last iteration, the Rome 4, is more extensively validated with a sensitivity 62.7% and a specificity of 97.1%[18]. The aim with the Manning criterion and the four iterations of the Rome criterion was to make IBS a positive diagnosis in less need of extensive testing for a firm diagnosis[17,19]. The validity of these diagnostic criteria has not been tested using conventional measurement of sensitivity and specificity in general population samples because there is no gold standard to allow independent confirmation of diagnosis, such as a biomarker[17]. To summarize, there is often considerable heterogeneity between studies, partly because of different diagnostic criteria being applied.

Table 1 IBS criteria, from Manning to Rome 4 [20 21 2]

The more of these symptoms, the more likely is that the patient's pain or altered bowel habit, or both, is due to IBS. The threshold for a positive diagnosis varies from two to four of the manning criteria [18 20 22]

Manning*	Rome 1	Rome 2	Rome 3	Rome 4
Abdominal pain that is relieved with a bowel movement	Continuous or recurrent symptoms of abdominal pain or discomfort, 3 months or more, that is:	Continuous or recurrent symptoms of abdominal pain or discomfort, 3 months or more, with two or more of the following, at least 25% of occasions or days:	Recurrent abdominal pain or discomfort (defined as an uncomfortable sensation not described as pain) for at least 3 days/month in the last 3 months, associated with two or more of the following	Recurrent abdominal pain on average at least 1 day/week in the last 3 months, associated with two or more of the following:
Pain associated with looser stools	Relieved with defecation	1. Altered stool frequency (>3movements/day or <3/week)	1. Improvement with defecation	1. Related to defecation
Pain associated with more frequent stools	And/or: associated with change in frequency of stool	2. Altered stool form (lumpy and hard or loose and watery)	2. Onset associated with change in frequency of stool	2. Associated with change in frequency of stool
Sensation of incomplete evacuation	And/or associated with a change in consistency of the stool	3. Altered stool passage (straining, urgency, tenesmus)	3. Onset associated with chane in the (appearance) of the stool	Associated with change in form (appearance) of stool
Passage of mucus		4. Passage of mucus		
Abdominal distension		5. Bloating or feeling abdominal distension		

The IBS diagnosis is based on the presence of characteristic symptoms and the exclusion of selected organic diseases. The general recommendation is to use Rome 3 as diagnostic criteria, and to add relevant diagnostic work-up for most relevant other potential disease after

history taking and physical exam. History taking on red flags such as weight loss, awaken in night by symptoms, fever in association with bowel symptoms, blood in stool is emphasized.

Additional testing often includes antibody testing for celiac disease and basic laboratory testing (blood counts, electrolytes, liver enzymes, c-reactive protein, stool cultures, occult blood, faecal calprotectin). Colonoscopy is not a part of the initial assessment, but should be performed if warranted by positive findings or if age appropriate. If colonoscopy is performed it is recommended to obtain pinch biopsies for ruling out microscopic colitis in IBS-D. SeHCAT screening to discriminate bile acid mal absorption from IBS-D is suggested, alternatively a therapeutic trial of bile acid sequestrate[5,23,24].

1.3 Treatment strategies

Opinions regarding the efficacy of treatments in IBS differ. Linedale C. and Andrew J 2015 claim in a narrative review that there are available effective therapies[25]. Craig O. 2017 acknowledges in a review that new promising treatments recently have become available, however a majority of patients who receive these treatments remain symptomatic. There are currently no medical treatments available for a cure of the disorder[26]. Older drugs and dietary interventions have been tested in small studies, with end points that would not be accepted by the present standards of the Food and Drugs Administration, whereas new drugs are more rigorously tested[6]. Treatments that involves the microbiome are assessed in the section ‘’targeting the microbiome’’ **on page XX.** A detailed description of all available treatments is beyond the scope of this thesis. For further reading: Linedale C. and Andrew J. 2017 provide a stepwise approach to the management of IBS, including medical and non-medical advice[25]. Craig O. 2017 summarise current and potential new medical treatments, including a brief overview of their mechanism of action[26].

The principal steps in managing IBS involves first establishing the diagnosis, diet advice and patient reassurance[26]. Reassurance by an explanation of the disease is found to reduce patients’ perceptions of the degree of impairment in daily function, whereas reassurance derived from colonoscopy to rule out organic disease in patients with IBS is short lived[27,28]. In terms of medical therapy, the usual approach has been to start an antispasmodic together with a laxative or an antidiarrheal depending on the predominant bowel pattern.

If first line therapy fails, antidepressants modulate pain perception and may treat coexistent psychiatric illness. It may also be beneficial because of the potential role of the brain-gut axis and altered central processing in IBS (further discussed in the section on the pathophysiology

of IBS)[26]. Hypnotherapy provided adequate relief in a multicentre randomized controlled trial including all subtypes. However, it is not clear whether hypnotherapy provides relief by educating patients better coping strategies, or by actually reducing the severity of the disease[29].

Second line therapy includes drugs designed to target serotonin receptor subtype antagonist or agonist, for IBS-D and IBS-C respectively[26,30]. This is of special interest to the thesis because gut bacteria also influence the serotonin signaling[31]. More than 90% of the body's serotonin is synthesized in the gut, where serotonin activates as many as 14 different serotonin subtype receptors located on enterocytes. Serotonin regulates diverse functions, including immune function, enteric motor and secretory reflexes (where the vagal nerve is involved)[32]. Although well documented efficacy, the drugs are not available in Norway because of concerns regarding the safety profile[33].

1.4 Natural history of irritable bowel syndrome

Studies on the natural course of IBS have the same methodological issues as the prevalence and incidence studies. IBS is a chronic disease that fluctuates in time in terms of change in clinical subtype, frequency and intensity of abdominal pain.

Patient reported symptoms associated with a higher disease burden are abdominal pain, bloating, bowel difficulties, limitations on diet and eating, concerns over disease and extra-intestinal symptoms such as myalgia. Abdominal pain is the most dominant feature. Elderly often report milder IBS, whereas women report more severe[3]. The quality of life in IBS is impaired, and the cost for society substantial with frequent health care seeking, absence from work and drop out from education[14]. One study found patients willing to give up 10-15 years of their life expectancy for an immediate cure[14].

IBS is associated with increased heath care seeking behaviour. However, only 17% of IBS meeting the former Manning criteria was found to seek medical advice in a 10 years follow up population screening program for Helicobacter pylori[34]. Approximately two thirds of patients with an IBS diagnosis from primary care are referred to secondary care, hence the increased health care seeking behaviour[35]. After 10 years 50-70% of patients report persistent symptoms[17,36]. Post infectious IBS (IBS with the prodrome of infectious gastroenteritis) have the best prognosis for complete spontaneous remission[5]. Currently, most patients treated for IBS remain symptomatic, and many medications are not suitable for use in all patients[26].

IBS is associated to both functional and organic disease. In active and remission phase of inflammatory bowel disease, 35% and 44% meet the criteria for IBS, respectively[37]. In addition, IBS is found in coeliac disease, idiopathic bile acid malabsorption and microscopic colitis[4]. IBS is associated to other functional disorders such as chronic fatigue syndrome, fibromyalgia, chronic headache, pelvic and mandibular joint pain syndromes[17,38]. This will be more thoroughly discussed in the final sections of the thesis. It is however established that there is a considerable overlap between IBS and functional dyspepsia (another gastrointestinal functional diseases)[39].

1.5 Pathophysiology of IBS

1.5.1 Genetics

The genetic risk spans from complex polygenic conditions with combinations of common variants, to cases with single variants associated with specific subtypes[40–42]. Twin studies estimated the genetic heritability in IBS between 22-57%[43]. Gene polymorphism is observed in relation to gut epithelial barrier function, neuronal function and visceral hypersensitivity[44]. Missense mutation in SCN5A is found in about two percent of IBS, and is most common in IBS-C. The *SCN5A* encodes the α-subunit of the voltage-gated sodium channel, suggesting benefits from antiarrhythmic drugs to patients with this mutation[42]. Gene variants involved in the serotonin pathway correlate to the clinical response of drugs that act on serotonin sub receptors[43]. An association of genetic polymorphism in tryptophan hydrolase 2 and fatigue is found in women with IBS[45]. Investigation of epigenetic changes in IBS is in its infancy. Animal studies have shown that visceral hypersensitivity can be transferred across generations, dependent on maternal care. Epigenetic changes associated to intestinal permeability, visceral sensitivity and serotonin receptor genes are observed[43].

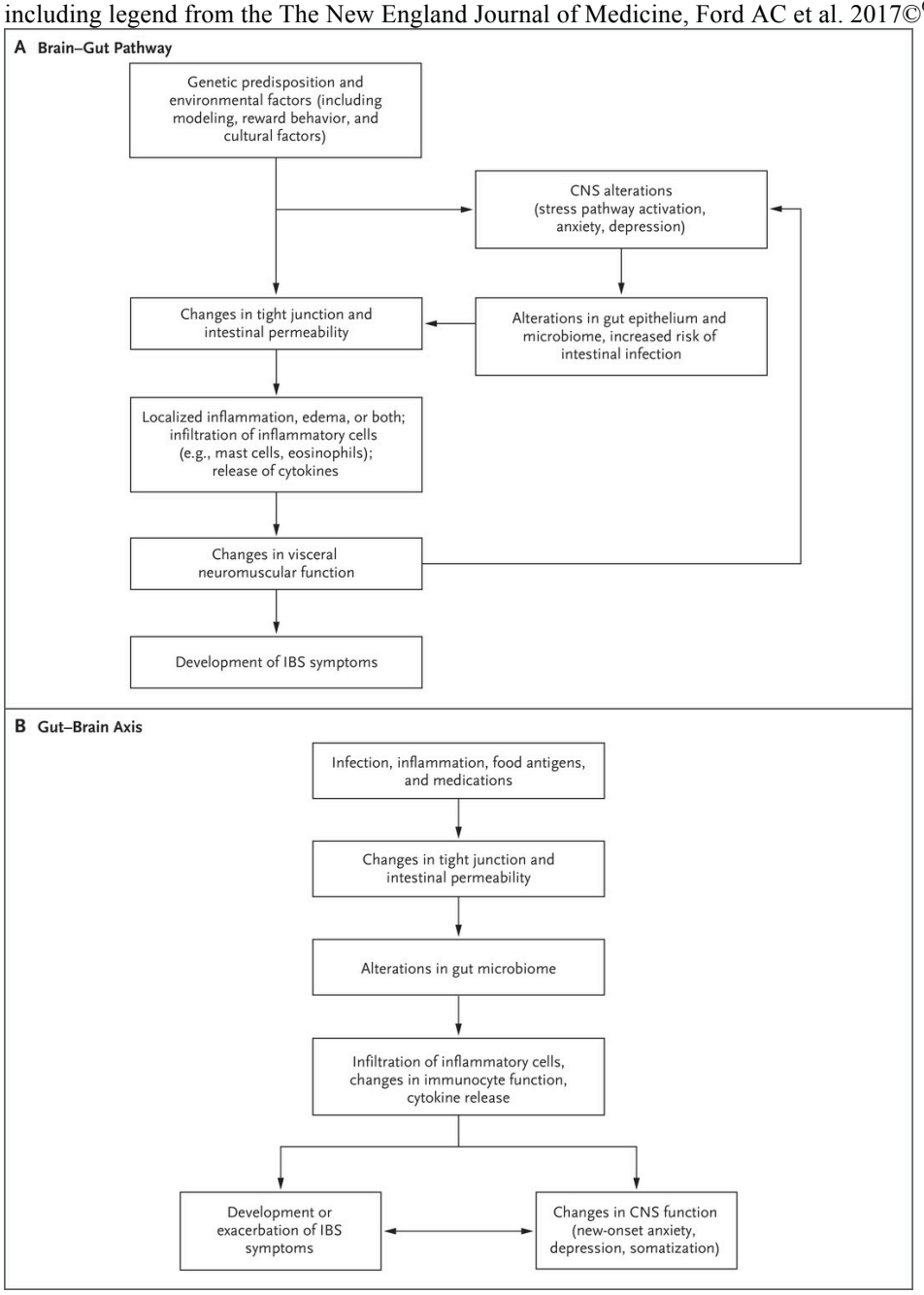
1.5.2 Microbiota-gut-brain axis

In neuroscience there has been a shift in paradigm, targeting the microbiome can modify central processing and cause changes in mood and behavior[46]. Microbiome refers to the genes contained in the organisms compromising the microbiota, and the microbiota refers to the bio-ecological community composed of multiple symbiotic, commensal and even pathogenic microorganisms in a community (i.e. the gut). Traditionally IBS has been thought of as brain gut disorder. Genetics and environmental factors early in life predispose to IBS and cognitive, behavioral, emotional and biological/physiological factors interact to precipitate and perpetuate symptoms and contribute to disability[3,47]. However, the traditional point of view is challenged by findings of bidirectional pathways for communication between microbiota and

the CNS. In this context the bidirectional communication between the microbiota, gut and the brain is frequently referred to as the microbiota-gut-brain axis[48–50]. Perturbation in this axis may influence central processing and autonomic functions such as visceral sensitivity, intestinal motility and permeability. The bidirectional relationship offers a potential pathophysiological framework to explain gastrointestinal and psychological disturbances in a heterogeneous patient group, but also represent a challenge in establishing causality. The field is constantly evolving with revisions and new findings. Figure 1 is an overview of the suggested interplay in the microbiota-gut-brain axis. Therefore, it is attempted to give a brief overview of the microbiota-gut-brain axis and its possible role in IBS in the following sections.

Figure 8 Pathogenesis of IBS

IBS has traditionally been thought of as a brain–gut disorder (Panel A). In susceptible persons (e.g., those with a genetic predisposition or exposure to environmental factors), an abnormal stress response, in combination with psychological distress (e.g., anxiety, depression, or somatization), and an infectious or inflammatory response may alter intestinal permeability and initiate a cascade of events (e.g., infiltration of inflammatory cells, localized edema, and release of cytokines or chemokines) that results in the development of IBS symptoms. Recent data show that immunocytes may play an important role in some patients. Coexisting depression, somatization, and catastrophizingmay also mediate changes in gut permeability, the immune system, and the microbiome, leading to the development of IBS symptoms. The presence of IBS symptoms may exacerbate symptoms of anxiety, depression, or somatization, further intensifying the gastrointestinal symptoms. Emerging data show that in up to half of patients with IBS, gastrointestinal symptoms develop first, with subsequent development of mood disorders (Panel B). Changes in the gut microbiome and the release of inflammatory mediators may be responsible for the central nervous system (CNS) disorders that arise after the development of IBS symptoms. The ensuing psychological distress may further exacerbate IBS symptoms. This is a modified version of an illustration including legend from the The New England Journal of Medicine, Ford AC et al. 2017©[6]



1.5.2.1 The HPA axis

The CNS can modulate the gut microbiota through executive pathways that determine gastrointestinal motility and secretion, intestinal permeability and gut immune response. Signaling pathways for these functions are mediated through the enteric nervous system via sympathetic and parasympathetic branches of the autonomic nervous system, as well as via the hypothalamus-pituitary-adrenal (HPA) axis[51]. Exposure to perinatal stress in animal models predisposes to the development of visceral hypersensitivity, compromise intestinal permeability, increased HPA axis response and anxiety like behavior[52]. An exaggerated stress response with increased levels of corticotropin-releasing factor is associated to exacerbation of GI-symptoms in patients with IBS[53]. Early adverse life events refer to traumatic experiences during childhood, encompassing physical, sexual, or emotional abuse, as well as discordant relationships with primary caretaker, or loss of parent. An association between developing IBS and experiencing early adverse life events is found, particular in women. Although, targeting an exaggerated HPA axis by blocking the corticotropin-releasing factor did not improve IBS symptoms in women with IBS[54].

1.5.2.2 Two-way communication

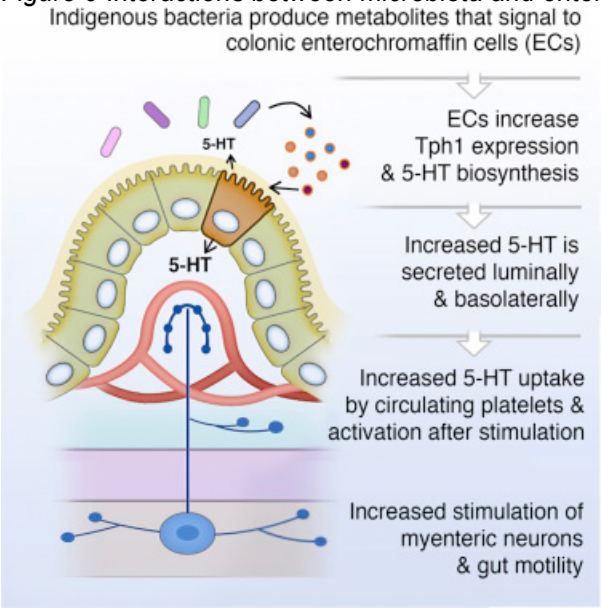
Central in the bidirectional communication is the vagal nerve, a component of the parasympathetic nervous system. With 80% afferent and 20% efferent nerve fibers it is able to sense the microbiota and transfer gut information to the CNS where it is integrated. The CNS can then generate an adopted or inappropriate response in terms of change in mood, behavior, and relay a response through efferent fiber in the autonomic nerve system and/or the HPA axis[31].

The vagal nerve is a key regulator of motility, secretion and food intake through the afferent sensory function and efferent executive functions. Under normal physiological conditions a balance between the parasympathetic nervous system and HPA axis is observed. This adapted homeostatic regulation couples high vagal tone to low cortisol levels[31]. A low vagal tone has been observed in IBS, and a lack of corresponding decrease in epinephrine and cortisol levels in response to an increase in vagal tone[55,56].

Vagal afferents are triggered directly by bacterial metabolites, or indirectly through serotonin and gut hormones from entero-endocrin transducer cells in the gut epithelial lining[31]. Entero-endocrin cells are scattered between the epithelial cells facing the gut lumen. These cells have specialized microvilli that project in to the lumen and function as sensors for the gut content. The gut hormones released by these cells regulate gut motility, cell proliferation, secretion,

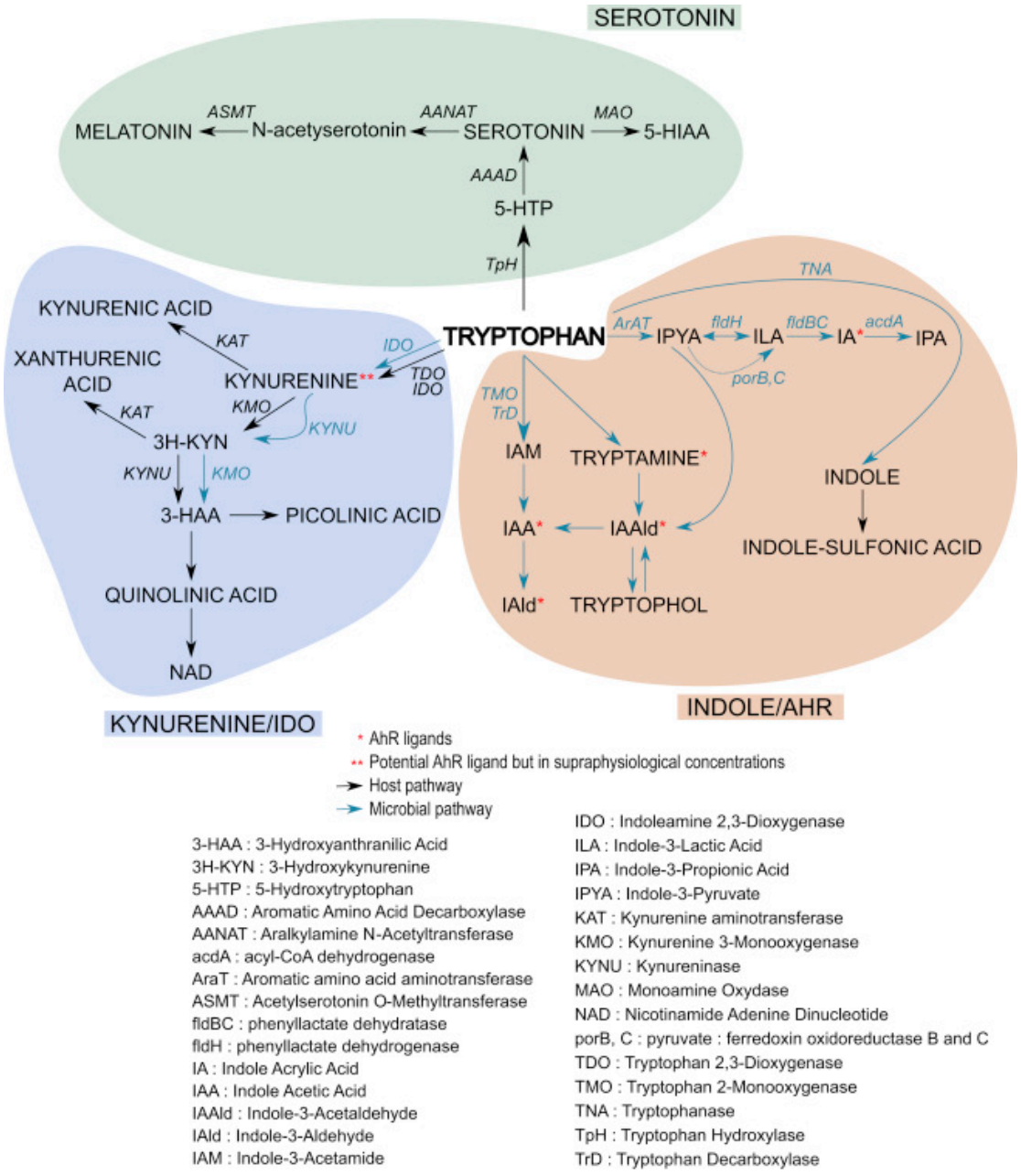
absorption, visceral sensitivity, local immune defence, cell proliferation and appetite through paracrine (act on nearby structures) or endocrine (enter the blood stream to act on more distant structures) mode of action. In IBS a lower density of endocrine cells, and their respectively progenitor cells is found both in the large and small bowel. It is observed that both adherence to a diet low in certain short chained carbohydrates, and changing the bacterial composition trough infusion of faecal suspension from a healthy donor to the gastro intestinal tract of patients with IBS, changes the density of endocrine cells in the large and small intestine[57].

Figure 9 Interactions between microbiota and enterochromaffin cells. ***©Cell, Yano et al. 2017***[58]



Tryptophan is an essential amino acid, and serotonin is a product in one of three pathways for the metabolism of tryptophan, shown in figure 3. In an animal model, spore-forming microbes from healthy mouse and human microbiota were observed mediating effects on serum, colon and faecal serotonin levels in an inducible and reversible manner [58] (shown in figure 2). In the tryptophan metabolism the kynurenine pathway also play a critical role in inflammatory mechanisms, immune response and neurobiological functions. IDO1 is a rate-limiting enzyme in the kyurenin pathway, and the microbiota play a key role in stimulating IDO1 activity. Kyurenin is increased in serum of IBS patients, and peripheral IDO1 activity is observed positively correlated with IBS severity[59].

*Figure 10 Host and microbial metabolism of tryptophan through the serotonin, Kynurenine and Indole pathway. ©**Agus et al. Cell host microbe 2018**[59]*



1.5.2.3 Microbiota and the immune response in IBS

The micobiota can induce a pro inflammatory state that involves local inflammation in the gut, impairment of gut epithelial integrity, neuroinflammation and induce pro and anti-inflammatory cytokines in the systemic circulation[60]. The neuronal and hormonal communication pathways involved drives diverse CNS regulated components of the inflammatory response including anhedonia, depression and mild cognitive impairment[60]. Improvement in IBS, associated to normalization of anti-inflammatory to pro-inflammatory cytokines, is observed after intake of a bacterial supplement[61]. Short chained fatty acids, and other metabolites from bacterial fermentation are a source of energy for gut enterocytes, and

influence the vagal nerve and central processing[31,62]. Altered colonic fermentation and functional output of short chained fatty acids is observed in IBS[63]. In addition, bacterial cell compounds such as lipopolysaccharides is found to be drivers of the immune response. Increased levels of lipopolysaccharide antibodies, and a correlation between flagellin antibodies and anxiety, is found in IBS[64].

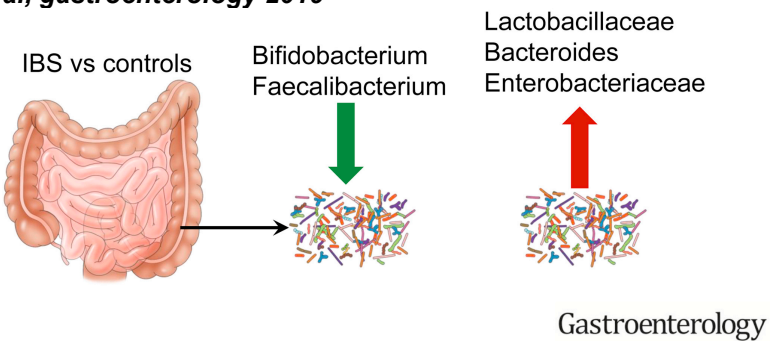
In IBS a low-grade inflammation with activity of both the innate and the adaptive immune response is found. Mast cells are suggested to play a critical role, particular in IBS-D. In addition, the cytokines and chemo attractants in IBS differ from healthy controls[65,66]. Increased intestinal permeability caused by immune activation is linked to diarrhoea and pain severity, suggesting that this mechanism might have a role in symptom generation in IBS[24]. Interestingly, a study showed that hypersensitivity to colonic distension of IBS patients can be transferred through infusion of faecal suspension from IBS to rats. However, no changes in epithelial cell permeability or density of mast cells were observed[67]. Another study where faeces from IBS patients were transferred to rats by faecal suspension demonstrated faster gastrointestinal transit, intestinal barrier dysfunction, innate immune activation, and anxiety like behaviour[68].

1.5.2.4 Microbiota in IBS

In the microbiota-gut-brain axis, changes in the microbiota can be cause or consequence (figure 1). Shared alterations and a core dysbiosis in gastro intestinal disease is suggested[69]. Diversity in IBS is often based on an abundance of microbes at different taxonomic levels, although several different diversity indexes exist. Diversity is suggested to predict gut health[70]. Although findings differ between studies, loss of diversity is not established as a signature of IBS[71]. This is further supported in one study, where the effect of donor FMT and placebo was compared. Donor FMT increased the diversity, but association between diversity and symptom improvement was negative when the groups was compared[72]. A specific microbiota profile in IBS is not found. However, an alternation in the microbiota when compared to healthy controls is evident, and suggested as a hallmark, even though there is considerable heterogeneity between studies[71,73]. In a systematic review from 2019 Pittayanon et al. found an increase in the Family Enterobacteriaceae (phylum Proteobacteria), family Lactobacillaceae, and genus Bacteroides, whereas uncultured Clostridiales 1. order, genus Faecalibacterium (including Faecalibacterium prausnitzii) and genus Bifidobacterium were decreased in IBS (figure 4)[71]. Conversely, a review of the microbiota in IBS from 2018 by Rodina-Janeira et al found a reduction in the relative abundance of lactobacillus, showing

there is considerable inconsistency between findings in studies.

Figure 11 Main findings when the microbiota in IBS is compared to healthy controls. ***©Pittayanon et al, gastroenterology 2019***[71]



Function can be assigned to the bacterial profile and includes; Increase in families (Enterobacteriaceae) containing strains with several pathogenic bacteria that may reflect previous infections. Increase in families (Lactobacillace) involved in colonic fermentation associated to abdominal pain and bloating. Increase in genus (Bacteriodes) with enterotoxigenic strains that affect the microenvironment, colonic mucosal production, intestinal motility and cause abdominal pain and diarrhea[71]. In addition, methane producers are found lower in IBS-D and higher in IBS-C. Methane is observed to have anti-inflammatory effects and is associated with intestinal transit time[74]. One study that combined taxonomic data with functional analysis identified a microbiota signature in IBS negatively associated to microbial richness, exhaled methane (CH4) and the enterotypes Clostridiales or Prevotella species. The signature was also associated to the severity of IBS symptoms[73].

1.5.3 Targeting the microbiota in IBS

Treatment strategies that involve the microbiota provide symptom relief in IBS. These include diet, probiotics and FMT. Use of antibiotics has also shown relief of symptoms. Best documented is the minimally absorbed antibiotic Rifaximin[75]. However, a recent study did not find any effects of Rifaximin on the fecal microbiota, organic acid extraction, nor the intestinal permeability in irritable bowel syndrome[76].

1.5.3.1 FODMAP

Diet restriction of short-chained fermentable oligo-, di-, mono-saccharide and polyol (FODMAP) provide relief of symptoms and improvement in quality of life in IBS[77]. Within the first 12 weeks 50-80% show symptomatic improvement from low FODMAP diet. The long-term effect is uncertain, but a lasting effect is observed. Bifidobacteria, suggested important to colonic health and reduced in IBS, is found in an even lower abundance when undertaking the low FODMAP diet[78].

The mechanisms for the effect of low FODMAP are not completely understood. FODMAP increase the osmotic drive and small intestinal water volume, which is thought to cause distention and abdominal pain in IBS with visceral hypersensitivity. However, a correlation between peak small intestinal water and symptom exacerbation is not found. It is unlikely that the relatively small increase in water volume is enough to cause diarrhea or loose stools. The FODMAPs also induce colonic fermentation, leading to accumulation of gas. This is also thought to provoke symptoms through distention and visceral hypersensitivity. Alterations in microbiota is observed, and changes in colonic fermentation pattern, with less gas production after initiating low FODMAP diet. In addition, a higher abundance of hydrogen using species, altered colonic fermentation with changes in the functional output of short-chained fatty acids, normalization of serotonin cell density and normalization of stool lipopolysaccharides is observed in patients on low FODMAP diet. Urinary metabolites, that include histamine – a modulator of inflammation and immune function, can discriminate high from low FODMAP diet[78]. A recent study found reduced levels of inflammatory cytokines, altered gut microbiota profile and, reduced levels of short-chain fatty acids associated with symptom relief from low FODMAP diet[63].These findings show associations between symptom severity in IBS, diet, alternations in the gut flora and signaling molecules (cytokines and short chained fatty acids) involved in the microbiota-gut-brain-axis. For more details of the suggested mechanisms to symptom relief by low FODMAP see Staucher et al 2017[78].

1.5.3.2 Probiotics

There is evidence for the effect of probiotics in IBS[75]. The strains and the composition of strains combined differ in clinical trials. In probiotics that combine strains the effect may be additive or canceled out. In addition, the estimated number of viable bacterial cells is often not the same in different formulas. Thus, reviews and meta-analysis that suggest benefits of a single or particular combinations of species or strain should be read with caution as many probiotic studies are not comparable[79]. Under these notions, the mechanisms for the effects of probiotics remain speculative. Studies has suggested that certain probiotics has the ability to modify expression of pain receptors in the gut of both mice and humans, normalize interleukin levels and reduce depression scores in IBS[75].

1.5.3.3 Faecal microbiota transplantation

Faecal microbiota transplantation (FMT) involves infusion of a faecal suspension from a healthy individual into the gastro intestinal tract of another person to cure a specific disease. The first report of a FMT effect in IBS was in 1989, in an issue of the Medical Journal of

Australia, by Thomas Borody with the heading ‘’bowel flora alteration: a potential cure for inflammatory bowel disease and irritable bowel syndrome’’. In a commentary to editor he hypothesised that alterations in bowel flora could cause IBS and IBD, and that FMT could restore a healthy flora and cure or cause symptom relief in a subset of IBS and IBD patients. In support of his hypothesis he published a case series of 55 mixed IBD and IBS patients where 20 reported cure of disease and 9 relief of symptom. Further details on the characteristics of the case series were sparse[80]. The first double blind placebo controlled trial is part of the present thesis, showing a positive short-term effect of FMT in IBS (paper 2). Recently four additional randomized controlled trials assessing the effect of FMT in IBS were published. Results are not consistent and this will be further discussed in the final section of this thesis.

1.5.4 Disturbance of the microbiota – cause or consequence?

The gut-microbiota-brain axis provides a theoretical framework that has the potential to merge diverging findings in IBS pathophysiology. Indirect and direct bidirectional communication between the microbiota, gut and CNS allows for several mechanistic explanations to the same set of symptoms without necessarily being contradictive. IBS is a heterogeneous disorder, however subgroups may share underlying pathophysiological mechanisms[4,6]. This implies that a disturbance of the microbiota may be a cause in one and a consequence in another subgroup. A third subgroup may entail patients where the synergy from altered central processing and disturbance of the microbiota cause the rupture of the disorder. Because of the heterogeneity and possible pathophysiological sub-entities that extends beyond the traditional phenotypic subtyping one cannot expect any therapeutic approach to be universally successful[79]. Research that pinpoints causal mechanisms will have a great impact on how patients are selected in clinical trials and treated in the future[4,6].

1.6 Summary of introduction

IBS is a functional disorder, where abdominal pain or discomfort associated with change in bowel habits, is the main characteristic. Currently IBS, is subtyped based on predominant bowel habits. Because the IBS diagnosis is symptom-based, and the diagnostic criteria have been changing over time, prevalence, incident and natural history of IBS remain elusive. IBS is a chronic disorder, the prevalence is often estimated to be about 10% and there are no treatments that offer cure for the disorder. The effect of the treatments for symptom relief is still controversial. Paper 2 was the first study to assess the effect of FMT in a double blind placebo controlled trial. A disturbance in the microbiota-gut-brain axis is suggested to be

involved in IBS pathophysiology, where alterations in the gut microbiota and central processing are found. However, it remains to determine what is the cause and effect. IBS is a heterogeneous disorder, so it is likely that what causes the rupture differ between individuals. If causality can be determined we are more likely to see interventions, in treatment and research, directed towards subgroups where the gut, the CNS or both is the cause for the rupture of the disorder.

2 Aims of thesis

General aims

The general aim of this thesis is to evaluate the effects of a single colonoscopic delivered donor vs placebo FMT in IBS-D and IBS-M. Our hypothesis was that FMT alleviate symptoms in IBS-D/M by restoring a healthy gut flora.

Specific aims

- To evaluate the point prevalence of uncovered microscopic colitis in the study population diagnosed by the Rome 3 criteria for FMT (paper1)
- To evaluate the effects of a single colonoscopic delivered donor FMT vs placebo on:
    - bowel related complaints in IBS-D/M by the Irritable bowel Symptom Severity Score (IBS-SSS) 3 and 12 months after treatment (paper2)
    - quality of life in IBS-D/M by the IBS-related Quality of Life (IBS-QoL) 6 and 12 months after treatment (paper3)
    - fatigue in IBS-D/M by the Fatigue Impact Scale (FIS) 3, 6 and 12 months after treatment (paper3)

3 Material and methods

3.1 Study design and patients

This was a double blind, randomized, placebo-controlled, parallel-group, single-centre trial designed to evaluate the effects of a single dose FMT in patients with IBS-M/D. Paper 2 and 3 evaluate the effect of FMT, while paper 1 is a cross sectional study where results are extracted from the patient cohort in the trial.

The study was performed at the University Hospital of North Norway, Harstad, Norway in collaboration with Sjøkanten Legesenter, Norway, Harstad (a centre for general practice). The

study was approved by the Regional Committee for Medical and Health Research Ethics of North Norway (2013/971), and conducted according to the Helsinki Declaration with funding from HelseNord (SFP1210-14) and the Norwegian Center of Rural Medicine. (ePhorte 20132561) All participants provided written, informed consent.

Patients between 18-75 years of age with moderate to severe IBS-D/M (IBS-M as long as constipation was not the dominating symptom) and abdominal discomfort/pain at least once a week were eligible for inclusion. The IBS diagnosis and subtyping were based on the work-up screening and in- and exclusion criteria listed in table 2. Patients were included at Sjøkanten Legesenter when the IBS diagnosis was verified, and the screening did not raise suspicion of alternative explanation to the bowel complaints. We excluded patients with nightly symptoms because this is a common feature in microscopic colitis (MC)[81]. The FMT donors were also included after a complete workup at Sjøkanten Legesenter, Harstad. The donor workup, screening, in- and exclusion criteria are listed in table 3.

We also did pinch biopsies of all patients during the treatment procedure for a histological assessment of the colon mucosa with special attention to MC. Biopsies were not a part of the initial assessment for inclusion. A pathologist examined the pinch biopsies (fixed in 4% buffered formalin and embedded in paraffin, cut in 3,5 µm thickness and stained with haematoxylin and eosin dye).

Included patients were randomised to a single donor FMT or placebo (autologous FMT), administrated in the right colon through the working channel of a colonoscope. After receiving allocated treatment, participants were discharged without any further observation unless immediate adverse effects were evident. Participants were given contact information to one of the study investigators to report adverse effects after treatment. Patient reported outcomes, faecal sampling and pinch biopsies obtained during the study are outlined in table 4.

Table 2 Screening work-up, in- and exclusion criteria for IBS participants

Inclusion criteria	Exclusion criteria	Work-up screening
IBS-D/M (IBS-M as long as constipation was not the dominating symptom) by the Rome 3 criterion)	Nocturnal abdominal pain or long-lasting abdominal pain with no variability	Past and present medical history and weight
Abdominal pain/discomfort at least once a week	Severe kidney failure, cardiac disease or pulmonary disease	Blood haemoglobin, complete blood-cell count, erythrocyte sedimentation rate, creatinine, sodium, potassium, C-reactive protein, aminotransferases, alkaline phosphatase, γ-glutamyltransferases, albumin, vitamin B_{12}, folic acid, ferritin, glycated haemoglobin, anti-tissue transglutaminase IgA, total IgA and IgE, thyroid-stimulating hormone and thyroxine.
Age 18-75	Immune deficiency or use of immuno-modulating medication	
IBS-SSS score ≥175	Assessed not being able to adhere to the tasks they were to perform as participants	
	Suspected alternative disease in the screening work up for inclusion	Faecal occult blood, pancreatic elastase, calprotectin and pathogenic bacteria (including toxin-producing Clostridium difficile)
	Food allergy	

Table 3 Screening work-up, in- and exclusion criteria for donors

Inclusion criteria	Exclusion criteria	Work-up screening
Body mass index ≥18	Use of antibiotics the past 3 months	Past and present medical history and weight
Age ≥18	High-risk sexual behaviour	Glycated haemoglobin, serology for HIV, Treponema pallidum and hepatitis A, B and C.
	Former imprisonment	
	History of inflammatory bowel disease, IBS, colorectal polyps, cancer, immunosuppression, obesity, metabolic syndrome, atopic skin disease or chronic fatigue	Faecal tests for Helicobacter pylori antigen, viruses, calcprotectin and occult blood.
		Faecal microscopy for parasites, ova and cysts
		Faecal cultures for Salmonella spp, Shigella spp, Campylobacter Spp, Yersinia spp, and toxin producing

		C. difficile

Table 4 Patient reported outcomes, faecal samples and pinch biopsy obtained during the study ***w=week, m=month including use of antibiotics during the follow up. *references to the appendices where the questionnaires and consent form are found***

	-4-2w	0	2w	1m	3m	6m	12m
IBS-diagnostic criteria and subtyping*	x						
Irritable Bowel Symptom Severity Score*	x	Intervention	x	x	x	x	x
Irritable Bowel Quality of Life*	x					x	x
Fatigue Impact Scale*	x				x	x	x
Five days dietary record*	x					x	
Complete list of medications	x						x
Self assessment questionnaire*	x						
Faecal sample	x					x	x
Participants consent*	x						
Pinch biopsy		x					

3.2 Randomization, masking and treatment allocation

A study nurse at a different hospital (The University Hospital of North Norway, Tromsø), created a randomization list using a randomization website (randomization.com). The randomization sequence for each block of six (active to placebo in the ratio 2:1) was concealed in opaque envelopes. A study assistant with no other involvement in the study allocated the treatment in each block one to six hours before treatment. Based on the placebo transplants marked with study numbers, and the matching study numbers with randomized treatment in the envelope, active or placebo treatment was allocated to each consecutive participant in a block of six. Study investigators enrolling patients were involved in the follow-up, but did not participate in the allocation of treatment or assigning of allocated treatment. Personnel from a separate ward assigned allocated treatment, and had no other involvement in the trial. Both patients and trial personnel remained blinded until the last included participant completed the 12 months follow up. The donor FMT and placebo were similar in appearance and were prepared by the same procedures. An elaboration of the procedures for preparing donor FMT and placebo follows in the next section. Donor FMT

was either fresh or frozen donor FMT. It was predetermined to use fresh donor FMT until 30 participants in the first blocks were assigned fresh donor FMT, and then use frozen donor FMT for the remaining blocks. However, if fresh donor FMT were not available on a particular treatment day frozen would be applied instead. The point where we switched from fresh to frozen donor FMT was then delayed accordingly to maintain the ratio of fresh and frozen donor FMT 1:1. For un-blinding when the study was completed, and in case of adverse events during study, a sealed opaque envelope with the full randomization sequence was kept in a sealed in safe at the University Hospital of North Norway Norway, Tromsø, only accessible to study investigators trough to the study nurse that created the sequence.

3.3 Donor and placebo FMT treatment

3.3.1 Procedure for preparation of transplants

Placebo and active transplants were prepared as follow: 50-80 g of freshly delivered faeces were mixed with 200mL of isotonic saline and 50mL of 85% glycerol, homogenised in a blender for 60 s, filtered through a 0,5 mm mesh steel strainer, drawn on 50 mL sterile Luerlock syringes and sealed. Placebo was participants own faeces obtained four to two weeks before treatment. Fresh donor FMT was prepared one hour before the first FMT treatment procedure in each block. Frozen donor FMT were prepared and frozen (-40^{o}C) at least two weeks before treatment of a full block. A mix of faeces from two donors was used, however in unequal amounts each time the procedure for preparation of transplants was repeated.

3.3.2 Procedures for standardisation of transplants

The frozen transplants (frozen donor FMT and placebo) were thawed over night in a refrigerator, but without transforming to liquid. Forty-five minutes to one hour before treatment transplants (donor FMT (fresh and frozen) and placebo) were transferred to a water bath (12 ^{o}C) to ensure identical appearance and temperature.

3.3.3 FMT treatment procedure

A dose of 8 mg loperamide was administered orally 2 h before endoscopy to retain the transplant. The transplants were administered to the caecum through the biopsy channel of an endoscope after pinch biopsies for standard histology were obtained. When the transplants were administrated participants were in a supine position, and remained in this position for 15 minutes, before being discharged.

3.4 Endpoints

3.4.1 The prevalence of microscopic colitis

The main focus of paper 1 was to report the point prevalence of microscopic colitis in the pinch biopsies from the IBS D/M study cohort from the REFIT study. MC in the pinch biopsies was defined as either lymphocytic colitis (intraepithelial lymphocytes >20/100 epithelial cells in the histologic assessment), or collagenous colitis (same ratio between lymphocytes and epithelial cells, but an additional sub epithelial collagen band of at least 10 ųm). MC is defined by the presence of histological inflammation (collagenous of lymphocytic as described above) in the absence of definitive endoscopic or radiological abnormality[82]. Lymphocytic and collagenous colitis are possible differential diagnosis to IBS-D in particular[83].

3.4.1.1 Undiagnosed disease and low grade inflammation by Geboes score

We also assessed the frequency of additional undiagnosed disease in the IBS study cohort uncovered by the test battery during inclusion and the histological exam of the pinch biopsies. In addition an assessment of inflammation in the pinch biopsies by the Geboes Score was also performed. Geboes score is histopathological scoring system to evaluate inflammation in ulcerative colitis[84]. Since low-grade inflammation is suggested as a possible pathological sub entity of IBS we wanted to evaluate if the Geboes scoring system identified inflammation in the pinch biopsies. Geboes score evaluate aspects of mucosal injury seen in ulcerative colitis including crypt architecture, lamina propria chronic inflammation, lamina propria eisonophils, lamina propria neutrophils, intraepithelial neutrophils, crypt destruction and surface epithelial injury[85].

3.4.2 Relief in gastro intestinal complaints by the IBS-SSS

The primary endpoint of the REFIT study, evaluated in paper 2, was the proportion of responders with relief in abdominal complaints three months after treatment when active treatment was compared to placebo. Secondary endpoint was the proportion of responders 12 months after treatment. A responder was defined as a 75 point decrease in baseline IBS-SSS. In addition we did secondary analysis that evaluated the time course of the treatment effect, if there were any additional predictors that determined the treatment response, and which individual components of the IBS-SSS had an effect on the global score.

3.4.2.1 The irritable bowel symptom severity score

The IBS-SSS evaluates primarily the intensity of IBS symptoms during a 10-day period: abdominal pain, distension, stool frequency and consistency (by rating satisfaction with bowel habits), and interference with life in general. The IBS-SSS calculates the sum of these five items each scored on a visual analogue scale from 0-100. A 50-point reduction in IBS-SSS is considered indicative of a responder[3,86].

3.4.3 Improvement in quality of life and relief in fatigue

The secondary endpoints from the REFIT study evaluated in paper 3 was the proportion of responder with improvement from baseline in quality of life at 6 and 12 months and relief in fatigue at 3, 6 and 12 months after treatment when active treatment was compared to placebo. A responder in quality of life was defined as an increase of ≥13 points in total IBS-QoL score. A responder in fatigue was defined as a decrease of ≥20 in total FIS score. In the secondary analysis we evaluated the time course of the treatment effect, if there were any additional predictors that determined the treatment response, and which subdomains of the FIS and IBS-QoL had an effect on their respective global score.

3.4.3.1 The fatigue impact scale

FIS is a 40 item questionnaire that assess the individuals' attribution of functional limitations to their subjective experience of fatigue in an overall score with three subdomains (cognitive, physical and social fatigue)[87]. Each item is scored on a 5-point Likert response scale (0 = ''no problem'' 1 = ''small problem'' 2 = ''moderate problem'', 3 = ''big problem'' 4 = ''extreme problem''). Higher scores indicate increased level of subjective experienced fatigue. The Norwegian version of FIS is validated to assess fatigue in IBS[87]. A conservative measure of minimal clinical important improvement, validated in patients with multiple sclerosis, is a decrease of 20 in total score[88].

3.4.3.2 The Irritable Bowel Quality of Life

Quality of life was assessed using a validated 34-item IBS-Quality of Life questionnaire with seven subdomains (dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual and relationships). Each item is scored on a 5-point likert response scale (1 = ''not at all'' 2 = ''slightly'' 3 = ''moderately'' 4 = ''quite a bit'' and 5 = extremely''). Data were transformed to a sum score (range, 0-100). An increase of ≥13 point in IBS-QoL score is considered a minimal clinical important improvement[89].

3.5 Statistics

3.5.1 Power calculation

The power calculation for this trial is based on estimates regarding the trial's over all main endpoint, the between groups difference (donor FMT vs placebo) in meeting a responder criteria of 75 points decrease in the IBS-SSS three months after treatment. Only results from case reports and an open-labelled study was available to estimate the treatment effect[90]. Having very little data from previous studies to estimate the FMT effect, we considered that a treatment response of 50% in the donor FMT group would be satisfactory if the treatment were to be applied in real clinical practice. The placebo effect is high in IBS (47%), but recedes after 12 weeks, and short duration of treatment and few office visits has shown to lower the placebo response[9]. We therefore calculated with 10% responders in the placebo group three months after treatment. Consequently, with a placebo effect of 10% and a response rate of 50% in the donor FMT group 50 participants would be needed in a balanced two-group design (α=0,05; 1-β=0,80). To allow for dropouts, we initially planned to enrol 60 participants.

However, to account for logistical difficulties relating to the use of fresh donor faeces, we altered the protocol (before enrolment of participants) to add a further 30 participants to the active group to allow for the use of frozen donor faeces in addition to fresh.

3.5.2 Statistical analysis

In paper 1 the point prevalence of MC was determined by the frequency of MC in the pinch biopsies obtained during the treatment procedure. Thus, the study population in paper 1 included only the participants we obtained a pinch biopsy from.

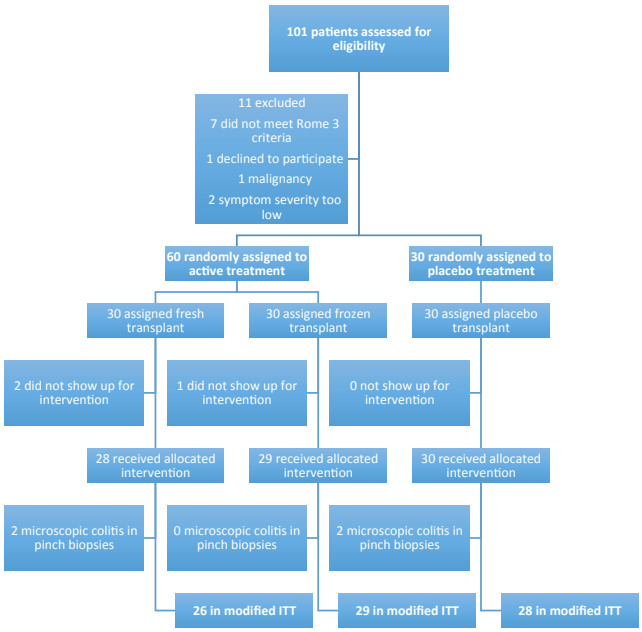
In paper 2 and 3 we did a modified intention to treat analysis excluding the participants that did not show up for treatment, and the participants with MC in the pinch biopsies. In the primary and secondary endpoints (paper 2 and 3) we reported the fraction of responders, when donor FMT and autologous FMT was compared in two by two contingency tables. In addition we did the secondary analysis in paper 2 and 3 by Repeated Measures Anova (RM-ANOVA) to assess the time course of the treatment effect and identify factors that predicted the treatment response (in addition to the FMT treatment). Finally, we did a doubly multivariate RM-ANOVA on the individual items of the IBS-SSS and the subdomains of the FIS and IBS-QoL to determine which components/subdomains had an effect on the global score. All statistical analyses were performed as two-sided tests, with a $p<0.05$ regarded as

significant. The computer software for the analysis was IBM SPSS Statistics version 24.0 and 25.0.

4 Results

The trial was performed between Jan 1., 2015 and November 30., 2016. A total of 101 patients were assessed for eligibility, and 90 with IBS-D/M were enrolled. In the final analyses 87 were included in paper 1 and 83 participants were included paper 2 and 3. For comparison in the modified intention to treat analysis of the FMT effect (paper 2 and 3) there were 55 participants in the active group (26 received fresh donor FMT and 29 frozen donor FMT) and 28 in the placebo group. Details regarding dropouts are provided in the trial profile, figure 5.

Figure 12 Trial profile



4.1 Paper 1

Baseline characteristics of the study population are found in paper 1. Four cases of lymphocytic type MC were identified in the pinch biopsies. MC was only found in IBS-D participants. Female gender, age more than 50 years old, autoimmune disease, smoking, proton pump inhibitors, selective serotonin reuptake inhibitors and NSAIDs are associated with an increased risk for developing MC[91–93]. Smoking, found in one of four MC cases, was the only risk factor we could identify besides age (three of four more were > 50 years old) and gender (three of four were female).

The point prevalence of MC in the age group more than 50 years old and with IBS-D was 27% (3 MC of in total 11 cases with IBS-D and age > 50 years). The point prevalence of MC in the total IBS-D and the IBS-D/M study population was 8,5% (4MC of 47 cases) and 4,6% (4MC of 87 cases) respectively. No pathological biomarkers from the initial test battery or other clinical findings could predict MC in this study. Forty-eight of the 87 participants were diagnosed with a colonoscopy before inclusion in the study. Of the four with MC; two participants had previously undertaken two colonoscopies, one participants had one colonoscopy before, and one did a colonoscopy for the first time when participating in the study. We do not know if pinch biopsies was obtained in the participants that had undertaken colonoscopy before the study.

During assessment for eligibility one participant that met the Rome criteria for IBS was excluded because of a pathological lymph node and was later diagnosed with malignancy. Additional testing with blood and stool samples did not lead to discarding of the IBS diagnosis in any of the participants, nor was additional unknown disease uncovered. Assessment of the pinch biopsies by Geboes score identified inflammation in the biopsies with MC and one additional case suggestive of ulcerative colitis.

4.2 Paper 2

In paper 2 the objective was to evaluate the effect of FMT in IBS-D/M on bowel related complaints by the IBS-SSS. The primary and secondary endpoint were the proportion, when active treatment was compared to placebo, with a 75-point reduction in the IBS-SSS 3 and 12 months after treatment, respectively.

Baseline characteristics of the study population were equally distributed in the active and placebo-group as shown in table 5. Presence of additional self reported functional disorder at baseline was associated to a higher IBS-SSS score (table 5). Point measurements of change in diet and medications, in particular the intake of FODMAPs, did not reveal any change from baseline and to three months after treatment (paper 2).

In the primary endpoint three months after treatment responders to FMT were 36 (65%) of 55 participants in the active treatment group versus 12 (43%) of 28 in the placebo group (p=0,049). Responders 12 months after treatment were 31 (56%) in the active vs 10 (36%) in the placebo group (p=0,075).

In a RM-ANOVA we used treatment group (fresh donor FMT, frozen donor FMT and placebo), IBS subtype and the presence of other functional disorders as predictors. Sex, age, psychiatric comorbidity, antibiotics, use of loperamide or change in FODMAP intake had no significant effect by itself (table 2 in the appendix to paper 2). The time course of the IBS-SSS scores during the whole study suggests frozen donor FMT to be more effective than fresh donor FMT and placebo. However, in the subgroup without other functional disorders the effect of both fresh and frozen donor FMT was similar, sustainable and more effective than placebo (paper 2, figure 2).

Finally, we did a post hoc analysis of the individual components of the IBS-SSS using the same predictors as in the RM-ANOVA analysis in a doubly multivariate RM-ANOVA. The two main complaints of IBS, pain and bloating, in addition to report of being content with bowel habits, were the most important contributors to improvement in IBS-SSS total score (table 3 in the appendix to paper 2).

One serious adverse event occurred: A patient in the active group was admitted and discharged on the same day because of self-limiting nausea and vertigo. Otherwise, adverse events were minor and self-limiting. One patient in each of the active and placebo group experienced soiling of transplant, one in the active and two in the placebo group experienced intermittent abdominal pain.

Table 5 Baseline characteristics and demographics

Data are median (IQR) or n (%), IBS=irritable bowel syndrome, IBS-SSS=irritable bowel symptom severity score, FODMAP=fermentable oligosaccharides, disaccharides, monosaccharaides, and polyols, MCII=minimally clinically important improvement, FIS=fatigue impact scale, IBS-QoL=irritable quality of life, *Self reported at inclusion; includes fibromyalgia, chronic fatigue syndrome, jaw- and pelvic pain syndromes, ŦSelf reported at inclusion, ŢCalculated from the 5-day dietary record. Score below MCII is not provided for the IBS-SSS as all participants had to have a a score of more than the MCII to participate

	Placebo (n=28)	**Active (fresh and frozen) (n=55)**	**Fresh (n=26)**	**Frozen (n=29)**
Age (years)	45 (34 to 57)	44 (33 to 54)	44 (35 to 54)	43 (26 to 54)
Sex				
Women	19 (68%)	36 (65%)	18 (69%)	18 (62%)
Men	9 (32%)	19 (35%)	8 (31%)	11 (38%)
IBS subtype				
IBS-M	15 (54%)	24 (44%)	12 (46%)	12 (41%)
IBS-D	13 (46%)	31 (56%)	14 (54%)	17 (59%)
Time with IBS (years)	10 (6 to 16)	10 (5 to 19)	15 (4 to 19)	10 (5 to 22)
Depression Ŧ	5 (18%)	9 (16%)	5 (19%)	4 (14%)
Functional comorbidity*	9 (32%)	14 (26%)	7 (27%)	7 (24%)
IBS-SSS at inclusion				
Total score	278 (223 to 254)	260 (226 to 313)	278 (233 to 354)	283 (224 to 330)
Score in IBS with functional comorbidity	345 (278 to 399)	315 (278 to 399)	259 (237 to 372)	319 (187 to 442)
Score in IBS without functional comorbidity	289 (216 to 293)	289 (228 to 295)	246 (246 to 280)	283 (228 to 327)

FIS at inclusion				
Total score	61 (32 to 96)	42 (16 to 78)	42 (26 to 79)	42 (16 to 80)
Score below threshold MCII	6 (21%)	15 (27%)	6 (23%)	9 (31%)
Score in IBS with depression	102 (79 to 129)	109 (56 to 123)	71 (25 to 123)	114 (90 to 144)
Score in IBS without depression	51 (20 to 80)	40 (15 to 60)	40 (22 to 64)	38 (15 to 58)
IBS QoL at inclusion				
Total score	46 (39 to 60)	60 (39 to 74)	61 (33 to 70)	58 (44 to 76)
Score below threshold for MCII	1 (4%)	2 (4%)	1 (4%)	1 (4%)
Score in IBS with functional comorbidity	38 (24 to 46)	56 (35 to 66)	60 (33 to 65)	52 (36 to 78)
Score in IBS without functional comorbidity	56 (44 to 66)	61 (44 to 76)	62 (32 to 79)	60 (49 to 75)
FODMAP before FMT (g/day) Ţ	0,0 (-4 to 4,7)	0,0 (-6,9 to 4,9)	-	-

4.3 Paper 3

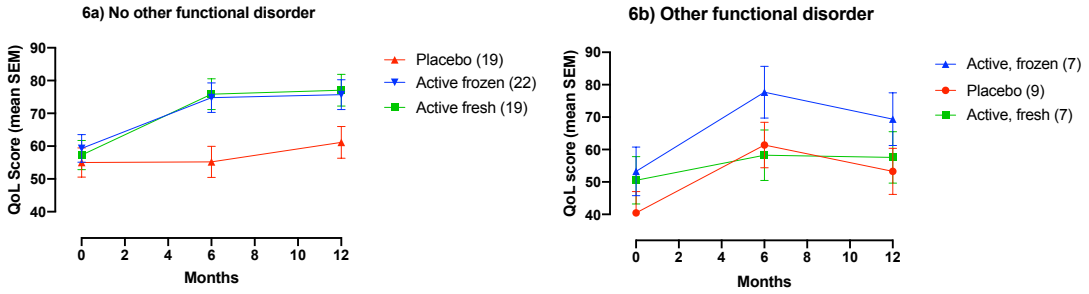
This paper has the same study population, with the same baseline characteristics as in paper 2 and table 5. There were no significant differences between active and placebo baseline FIS or IBS-QoL total score (table 5). Interestingly, there seems to be an association between baseline depression and fatigue, as the baseline fatigue score is twice as high in the subgroup with IBS and depression, compared to the subgroup with IBS and no self-reported depression before treatment. Same point measures of change in diet and medication was applied as in paper 2 and revealed no important changes in neither.

In the secondary endpoint were the effect of FMT on QoL was assessed we found a significant effect of FMT after 6 months, but not after 12 months, when active treatment was compared to placebo. Six months after treatment there were 47 (86%) responders in the active group vs 17 (61%) responders in the placebo group (Odds ratio (OR) 3,801; confidence interval (CI)=1,309-11,042 p=0.011). Twelve months after treatment there were 43 (78%) responders in the active group vs 17 (61%) responders in the placebo group (OR=2,319; CI=0,860-6,254 and p=0,093)

In the secondary endpoint where the effect of FMT on fatigue was assessed we found a significant effect of FMT after 6 months, but not after 3 and 12months, when active treatment was compared to placebo. Six months after treatment there were 19 (35%) responders in the active group vs 3 (11%) responders in the placebo group (OR=4,398; CI=1,175-16,468 and p=0,020). Three months after treatment there were 17 (31%) responders in the active group vs 5 (18%) responders in the placebo group (OR=2,058; CI=0,669-6,330 and p=0,203). Twelve months after treatment there were 17 (31%) responders in the active group vs 9 (32%) responders in the placebo group (OR=0,944; CI=0,355-2,511 and p=0,909).

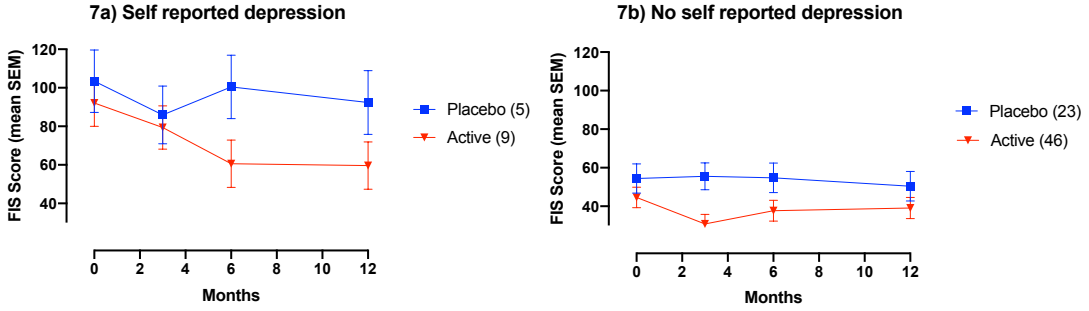
In a post-hoc analysis of IBS-QoL we did a RM-ANOVA with treatment group, IBS subtype and presence of other functional as predictors in a full factorial model, optimised by removing non-significant terms. Sex, age, psychiatric comorbidity (self reported depression), antibiotics, or use of loperamide did not have a significant effect by itself nor changed the conclusions of the model. The RM-ANOVA showed a significant treatment response in IBS-QoL adjusted for self-reported functional disorders at baseline (Partial Eta Squared (ηp^2)=0,112 and p=0,023) (paper3). Comparing the subgroups with and without additional self-reported functional disorders there are important distinctions in the treatment response (figure 6A and B). The subgroup without other functional disorder (figure 6A), given active treatment (fresh or frozen donor FMT), shows a profound response from baseline to six months that sustain to twelve months. Same effect is not found in the corresponding placebo group. The participants with other functional disorders (figure 6B) show a low and transient treatment effect in both active (fresh or frozen) and placebo group. Finally, we did a post hoc analysis of the individual components of the IBS-QoL using the same predictors as in the reduced RM-ANOVA model in a doubly multivariate RM-ANOVA (appendix to paper 3, table A3). The three subdomains, interference with activity, body image and relationships had a significant effect on the global score.

Figure 13 Time course of the treatment effect on IBS-Quality of life from baseline and until 12 months after treatment in IBS without (6a) and with (6b) additional baseline self-reported functional disorders. ***(Number of participants)***



In a post-hoc analysis of FIS we did a RM-ANOVA with treatment group (active (fresh and frozen combined) vs placebo) and depression as predictors in a full factorial model. Sex, age, IBS-subtype and other functional disorders did not have a significant effect by itself. The analyses show a significant treatment response in fatigue when adjusted for self reported depression at baseline (ηp^2=0,104 and p=0,005). There are distinct differences to the treatment response in the subgroup with and without self-reported depression at baseline (figure 7a and b). The subgroup with self-reported depression (7b) at baseline shows a treatment response that increase from baseline and to six months and sustains until twelve months, whereas the placebo response in the same subgroup is low. The subgroup without self-reported depression (7b) shows a treatment effect from baseline to three months that relapses and becomes indistinguishable from the effect in the corresponding placebo group. Finally, we did breakdown of the individual components of the FIS using the same predictors as in the RM-ANOVA analysis in a doubly multivariate RM-ANOVA (appendix to paper 3, table A5). All three subdomains (physical, cognitive and social) were important to the detected signal.

Figure 14 Time course of the treatment effect on Fatigue Impact Scale from baseline and until 12 months after treatment in IBS with (7a) and without (7b) baseline self reported depression. ***(Number of participants)***



5 Discussion

5.1 Validity of the results

The patient population in paper 1, 2 and 3 was moderate to severe IBS-D or IBS-M (without dominating constipation diagnosed) by the Rome 3 criteria. Compared to patients with mild symptom severity, patients with moderate to severe are more likely to use health care services, have a lower QoL, more fatigue and extra intestinal symptoms including mood disorders[3]. Thus, it is not certain that our results are valid in an IBS population with mild symptom severity.

The Rome 3 criterion, which was the current diagnostic criterion when we performed the study, has now undergone a revision. The new Rome 4 criteria only allows for abdominal pain with a frequency of at least one day per week the last three months. Rome 3 allowed abdominal pain or discomfort with a frequency of at least three days per month, for at least three months, during the last six months (table 1). Compared to the Rome 3, the new Rome 4 requires that the abdominal symptom is more intense (only allowing pain) and persistent (weekly instead of monthly pain). Since the Rome 1 criterion was established in 1989 and until Rome 4 in 2016, discomfort and pain has been juxtaposed (table 1). The validity of our results in a patient population diagnosed by the current Rome 4 criterion is uncertain. However, a determinant of severity is the intensity of the abdominal pain[3]. As our study only included moderate to severe IBS, abdominal pain (and not discomfort) is likely to have been a common complaint. In addition, patients had to report a frequency of the abdominal pain or discomfort at least once a week to be included. The reason for only including moderate to severe IBS with discomfort or pain at least once a week was to ensure responsiveness to treatment. In retrospect, participants were included by a modified Rome 3 criterion very similar to the new Rome 4 criterion.

5.2 Challenges in this trial

Challenges in clinical trials for IBS include insufficient understanding of disease mechanisms, high placebo effect and lack of specificity of symptoms. It is difficult to differentiate IBS from alternative disease. Biomarkers for diagnosis and monitoring of disease activity does not exist[94,95] Nor has diagnostic criteria in bowel motility or visceral sensitivity been described[96,97]. There is variation in intensity of symptoms[95]. Patients assessed during a period with aggravation of symptoms are more likely to report relief after treatment. In IBS it is observed a placebo effect of 47%, which stabilizes over a period of 2-5 weeks and lasts

approximately 12 weeks before starting to recede[9]. In a trial that randomly assigned patients with IBS to placebo, which they were told had ‘’mind-body self-healing’’ effects, or to no treatment, 59% of those assigned to the placebo reported adequate relief of symptoms, as compared with 35% of those receiving no treatment (p=0,03)[94]. High placebo response is associated to long duration of treatment and a greater number of office visits. Thus, trials should have a duration of at least three months for the placebo effect to diminish[9].

Because IBS is a heterogeneous disorder it is essential to capture which symptoms improve when a treatment is tested[98]. Currently there is no patient reported multi item instrument for primary measures of efficacy that is recommended by the European Medicines Agency (EMA) or Food and Drug Administration (FDA)[99,100].

There is no straightforward approach for a solution to these challenges, as demonstrated by a survey in 2013 where 39 international experts in IBS answered.

- 66% diagnosed IBS easily without too many test.
- 80% felt the need for new multinational valid criterion for diagnosing.
- 77% did not feel the Rome Criteria reflected the IBS in their own country
- 60% used their own clinical experience rather than a Rome criterion to diagnose IBS.
- 29% considered abdominal pain the most bothersome symptoms among IBS[101].

In the following it is discussed if the correct measures to exclude alternative disease in this study was taken (relates to paper 1) and if the appropriate endpoints to assess the effect of treatment was chosen (relates to paper 2 and 3).

5.2.1 Diagnosing IBS-D/M for FMT

The study population in paper 1 was mixed with previously diagnosed and undiagnosed IBS. However, a record of the exact distribution was not kept. The point prevalence of uncovered disease from the physical exam, history taking, colonoscopy, blood and stool test might increase in a study population where IBS is diagnosed for the first time. Benefits from extensive blood and stool tests in paper 1 were not found. The study population does not allow us to draw any conclusion whether an extensive base line assessment is beneficial in a patient population with only undiagnosed moderate to severe IBS assessed for FMT. The physical exam revealed malignancy mimicking IBS symptoms in one participant, which underlines the importance of a thorough physical assessment.

The finding of four cases with MC was not expected. To limit the number of MC in the study population we excluded patients with nightly abdominal symptoms as this is more common in MC than IBS[102–104]. Thus, we expected the point prevalence of MC to be lower in paper 1 than reported in other studies. In paper 1 the point prevalence of MC in non-constipated IBS and IBS-D was similar to previous studies (paper 1; 4,6% and 8,6% respectively). A prevalence of 1,5% and 6,1-8,3% is found of MC in non constipated IBS (IBS-D and IBS-M) and IBS-D respectively[102,105,106]. Because the odds of microscopic colitis is observed to be no higher in patients with IBS compared with other patients with diarrhoea, the value of routine investigations of MC in IBS is disputed[83,102,105,106].

The point prevalence of MC in paper 1 is more likely to underestimate than overestimate the frequency MC in the study population of paper 1. Firstly; MC only in the right colon is possible although not frequent[104,106]. We only obtained biopsies from the left and transverse colon. Second; a repeated colonoscopy with pinch biopsies may increase the diagnostic yield. In a retrospective study 30% of MC cases were identified in the repeat, and not the first, colonoscopy[104]. This may explain why three of four MC cases in paper 1 previously had undertaken colonoscopy, but without being diagnosed with MC. Conversely, in the same study 30% of cases identified as MC in the first colonoscopy, did not meet the criteria for MC in the second. Instead chronic inflammation was found[104]. We assessed all biopsies with Geboes score, but did not identify any cases with chronic inflammation in paper 1. Chronic inflammation in lamina propria, but not enough to fulfil the diagnostic criteria for MC is referred to as incomplete microscopic colitis. It is a suggested sub entity of MC and may benefit from treatment with budesonide[104]. Third immunohistochemical CD3 staining can increase the diagnostic yield of MC compared to the hematoxylin and eosin staining we diagnosed MC with in paper 1[107].

Additional investigations to ensure a correct IBS diagnosis depend on IBS subtype and concerning features in the patient history and physical exam. IBS symptoms in age > 50 years is often referred to as a concerning feature that prompts colonoscopy. However, the recommendations for doing pinch biopsies to rule out MC in this age group range from ‘’pinch biopsies should be performed[5]’’ to ‘’pinch biopsies should be considered[24]’’. We found a point prevalence of 27% in the subgroup of participants with IBS-D over 50 years of age (paper1). Results suggest that colonscopy with pinch biopsy for MC should be a routine investigation in this subgroup, however this needs to be confirmed in larger studies.

We aimed to exclude alternative disease to IBS symptoms by an extensive baseline work up (paper 1-3). However, we did not assess bile acid malabsoption by SeHCAT in the IBS-D patients. Bile acid malabsorption diagnosed by SeHCAT is found in approximately 1 of 4 IBS-D patients[108]. Bile acid malabsorption is probably under diagnosed in IBS[108,109]. A systematic review found 80% response rate to treatment with bile acid sequestrates in IBS-D patients with a positive SeHCAT test[109]. However, the validity of this study is questioned because of limitations in the study design[108]. We did not have the resources, nor did the hospital facilities have the capacity, to perform SeHCAT on all IBS-D patients within our time budget. It did not seem reasonable to request a therapeutic trial with bile acid sequestrates before participation in the study when the placebo response in IBS may be to 47%, and last until 3 months after treatment[9]. Responders to a test with sequestrates might be given a misleading diagnosis because of the placebo effect. We did not keep a record to show the distribution of IBS-D participants that had performed SeHCAT before enrolment to estimate proportion of cases with potential bile acid mal absorption.

5.2.2 Outcome measures

Current recommendations from EMA and FDA for the primary measure of efficacy in clinical trials with IBS is to use a composite endpoint with rating scales of abdominal pain and a subtype specific criterion that assess frequency or consistency of the stool. In clinical trials where two or more subtypes are included, or if the candidate treatment is not acting on one specific symptom or mechanism, a patient reported assessment of adequate relief is allowed. Anchoring of adequate relief by exploratory endpoints that assess important characteristics such as abdominal pain, bloating, stool frequency and consistency is recommended[99,100]. We assessed the FMT effect in both IBS-D and IBS-M, so the primary endpoint according to the EMA and FDA, should have been adequate relief. In the secondary endpoints abdominal pain, bloating, stool frequency and consistency should have been assessed.

In paper 2 our primary endpoint was symptom relief of more than 75 point assessed by the IBS-SSS. The IBS-SSS is a validated self-assessment tool for the severity in IBS and is shown responsive to treatment[3,86]. The 75-point decrease is a conservative measure to define responders, as a 50-point decrease is the validated limit for minimal clinical important improvement. However, this was an adjustment we made to overcome the anticipated high placebo response[3,86]. The IBS-SSS is not validated by FDA standards nor accepted as a primary patient reported outcome for clinical trials in IBS[99,110]. The IBS-SSS entail items that assess both pain, bloating and bowel habits. Post hoc analysis allowed us to assess each items

contribution to the global improvement by a doubly multivariate RM-ANOVA in paper 2. We assessed both the global effect of FMT on bowel habits, and also each symptom individually. This approach was an adjustment to the EMA or FDA recommendation and allowed us to assess both the global and item specific effect with one simple questionnaire (IBS-SSS).

In paper 3 we wanted to assess the effect of FMT on quality of life and fatigue. As the severity in IBS is a composite of intra and extra intestinal symptoms, we wanted to include one measure of the treatment effect that did not involve intestinal symptoms[3]. Fatigue, experienced as a bothersome symptom in ninety percent of IBS patients, is one of the most pronounced domains with decrements in health related QoL[111,112] and is moreover found as an independent predictor for referral to the secondary health care[112]. A link between IBS and chronic fatigue syndrome is suggested because of overlapping symptomatology[38,113]. To our knowledge this is the first trial in IBS that assess the responsiveness of fatigue to treatment in IBS (paper 3).

5.3 The effect of FMT in IBS

In paper 2 we found a significant effect on bowel related complaints by the IBS-SSS at 3 months (active 65% and placebo 49% responders, p=0,049), but not at 12 months (active 56% and placebo 36% responders, p=0,079). In paper 3 we found a significant effect on quality of life by the IBS-QoL at 6 months (active 85% and placebo 61%, p=0,011), but not at 12 months (active 78% and placebo 61% responders, p=0,093). In the same paper we also found a significant effect on fatigue by the FIS at 6 months (active 35% and placebo 11% responder, p=0,020), but not at 3 months (active 31% and placebo 18% responders, p=0,203) nor at 12 months (active 31% and placebo 32% responders, p=0,909).

The results show a corresponding and consistent effect of FMT on both bowel related complaints, quality of life and fatigue the first three to six months after treatment. The effect on fatigue shows a minor deviation with a significant effect at six, but not at three months after treatment. However, the study was not adequately powered and participants were not selected to determine the FMT effect on fatigue. It is also possible that the effect on fatigue is delayed compared to the effect on bowel related complaints. It would have been interesting to compare QoL with FIS and IBS-SSS score three months after treatment to determine if the peak effect on QoL corresponded with the peak effect on IBS-SSS or FIS. We did not want to increase the workload on participants with the QoL questionnaire three months after treatment, as they also had to do the five days dietary record at this time point.

In the post hoc analysis, frozen FMT was more effective than fresh donor FMT and placebo on bowel related complaints and quality of life (paper 2 and 3). This finding is most likely caused by additional functional comorbidity at baseline as a confounder when the effect between fresh and frozen donor FMT is compared. In the subgroup without additional functional comorbidity there is no difference between the effects of fresh or frozen donor FMT when compared to placebo (paper 2 and 3). Additional functional comorbidity, in addition to self-reported depression at baseline, is associated to the effect of treatment and will be thoroughly discussed in the following sections (paper 2 and 3). An important notion is that fresh and frozen donor FMT was not randomised for a comparison. Active treatment (fresh and frozen combined) was randomized to placebo (2:1) in blocs of six, and then we predetermined the use of fresh or frozen donor FMT in each block. In total we kept the ratio of blocks with fresh and frozen 1:1. Since fresh and frozen are not randomised caution should be made in drawing conclusions regarding the effect of fresh vs. frozen donor FMT in IBS.

In the literature results from clinical trials assessing the effect of FMT on IBS are diverging. A recent review and meta analysis in the American Journal of Gastroenterology concludes: ‘’Current evidence from RCTs does not suggest benefit of FMT for global IBS symptoms. Questions remain regarding the efficacy of FMT in IBS as well as the lack of a clean explanation on the discrepant results among RCTs in subgroup analysis’’. This review and meta analysis included 4 studies (paper 2 was one of the four) with 254 eligible participants. Heterogeneity of studies was significant ($I^2 = 79\%$)[114]. Important characteristics that may have an impact on the result differed between studies, including route of administration, total dose of donor stool used and procedures for processing donor transplants. A review and meta analysis more recently published includes the same four studies with one additional where FMT was administrated by colonoscopy. In the conclusion, attention is given to the fact that FMT administration by colonoscopy in two studies and nasojejunal in one has shown a positive effect, whereas the negative results are from two studies with capsulated FMT treatment; ‘’Fresh or frozen donor stool delivered via colonoscopy or nasojejunal tube may be beneficial in IBS. Larger, more rigorously conducted trials of FMT in IBS are needed’’[115].

There are many possible reasons to the difference in results between studies included in the meta analysis[114,115], making the comparison and assessment of a pooled effect controversial. Firstly, the density of the microbiota increase from the stomach to the colon[116]. In addition, the fermentation of FODMAPS takes place in the colon. This fermentation process is suggested to be involved in IBS pathophysiology[77,117]. Upper delivery of transplants, and

capsulated FMT, may lead to an increase of the bacterial population in the upper parts of the gastro intestinal tract and cause symptom aggravation. Considering the integrity of the mucosa of the large vs the small intestine, it is also possible that the chance for uptake of bacterial antigens is increased from upper delivery as the small intestine has a larger and more effective absorptive surface design for uptake[118]. In addition, pre-processing from passing through the digestive system may have an impact on how the transplant engraft and influence the colonic fermentation. The survival rate may differ between different taxa leading to changes in the effective profile delivered in the colon. In paper 2 and 3 we delivered the transplants to the colon so that the microbiota could engraft in its natural habitat without any pre-processing or -selection from passing through the digestive system.

Second, the glycerol used as a cryoprotectant in frozen FMT can alter the colonic microbiota[119]. In the two studies with capsulated FMT the glycerol content was respectively 30% and uncertain (capsules was provided by a non profit stool bank to the study so the processing of transplant is not described) respectively. In paper 2 and 3 we did as the current guidelines recommend for stool banking of frozen faeces, which is having a glycerol concentration of ten percent in the transplant[120].

Third, exposure to oxygen and freeze-thaw cycles has an impact on the viability and reduces the diversity of commensal taxa with capacity for biosynthesis of important anti-inflammatory metabolites[121]. The processing of transplants in the individual studies is not described in the meta analysis for a detailed comparison. However, it is reasonable to assume that pooled donor FMT with sequential freeze-thaw cycles and capsulated FMT have a viability of commensal taxa different from transplants processed within one hour after defecation, frozen and then thawed before FMT treatment. On the other hand, our post hoc analysis of the FMT effect did not show any difference in the FMT effect between fresh and frozen donor faeces (paper 2 and 3) in the subgroup with no additional functional disorders. In paper2 and 3 the freeze-thaw cycle was one and zero for frozen and fresh donor faeces respectively. This questions if viability of commensal taxa matters. Sterile faecal transplants can treat recurrent Clostridium difficile[122], which also show that viability of commensal bacteria is not necessary what determines the treatment effect.

Forth, the recommended dose to treat recurrent Clostridium difficile infection is 50g faeces in a single dose[123]. We considered this recommendation the gold standard, as recurrent Clostridium difficile infection is the only disease where a beneficial FMT effect is

undisputed. Recently, an amount of 25-30g colonic delivered faeces is shown effective in recurrent Clostridium difficile[120]. Amount of faeces and number of administrations differ between studies of FMT in IBS. Of the two studies with capsulated FMT one of the studies exceeded the recommended dose by far giving 144g faecal matter derived from 600g pooled donor faeces in 300 FMT capsules (25 capsules daily for 12 days). In this study there was a significant improvement on bowel related complaints in placebo compared to donor FMT group. Placebo capsules were made from saline, glycerol and food colouring. In the other study the total dose was 28g (in the meta analysis the total dose was 50g but this is wrong according to the original paper[124]) (25 capsules for three days), and there were no significant difference on the effect on bowel related complaints between the donor FMT and placebo group. Although speculative, this raise the question if there is a dose dependant effect. Donor FMT compared to placebo; a capsulated dose of 30mg had no effect, our study with 50-80g favoured colonoscopic donor FMT (paper 2 and 3), whereas 144g capsulated FMT derived from 600g donor faeces showed an improvement in favour of placebo. It is possible that the capsulated FMT derived from 600g faeces exceeded what the upper gastro intestinal tract can handle before an adverse effect occur. An very recent randomized controlled trial with IBS participants, receiving 30g or 60g of donor FMT by a nasjojejunal delivery, found a dose dependant effect in favour of 60g [125].

Fifth, the study populations are not the same. Participants are included based on different IBS-subtypes, and other criteria such as modified Rome criteria (paper 2 and 3) and low amount of butyrate-producing bacteria in the faecal samples[115].

Sixth, bowel lavage may cause alteration in the gut microbiota[127]. It is possible that bowel lavage before FMT has an effect on the short and long term engraftment process. In paper 2 and 3 we prepped the participants with bowel lavage before FMT. In at least one of the studies with capsulated treatment this was not done[115].

Seventh, our study has the highest sample size of the studies included in the meta analysis, but was barely able to detect significant differences between the active and placebo group on the primary endpoint, mainly because of a high placebo effect. Small sample size may have biased the individual studies in the meta analysis. In addition, FMT is not a standardised treatment as each donor has a specific microbiota. Only 14 of 664 genera conform to a core microbiome in the gut[71].

5.4 Predictors of an FMT effect

The RM-ANOVA analysis highlights that predictors in addition to treatment group (active or placebo FMT) determines the treatment response. This is an important finding, as the additional predictors points to subgroups with a sustainable treatment effect on both bowel related complaints, quality of life and fatigue.

On bowel related complaints (by the IBS-SS in paper 2) and quality of life (by the IBS-QoL in paper 3) additional self-reported functional disorders at baseline and IBS-subtype had an effect on the treatment response from baseline and to 12 months. Most evident is it that other self-reported functional disorders at baseline are associated to less benefits of FMT treatment for both bowel related complaints (paper 2) and quality of life (paper 3, figure 6b). Figures on how IBS-subtype predicted the effect is not provided because our sub analysis did not show a pattern that provided any further insights into effect of this predictor. In fatigue only self-reported depression at baseline in addition to treatment group (active or placebo) predicted the treatment response.

Other functional disorders which include; self reported fibromyalgia, chronic fatigue syndrome, jaw and pelvic pain syndromes are associated to more severe IBS[3]. These are conditions with poorly understood aetiologies, defined by symptoms that have a considerable overlap with IBS[17,38]. However, a study that compared patients with an IBS diagnosis to age and gender matched controls found no unique association between IBS and other functional disorders, but rather a general amplification of disease incidence in IBS[128]. If there is a shared pathophysiology, if functional disorders coexist on a continuum or if the symptoms just are overlapping requires further investigation[38].

Our results show that not having any self-perceived functional disorders other than IBS at baseline is associated to a profound and sustainable improvement in quality of life. A dysbiosis is suggested to be involved in both fibromyalgia and chronic fatigue syndrome[129,130]. The results were surprising because we thought the subgroup with additional self-perceived functional disorders should in particular benefit from FMT treatment. Either, this subgroup has a more severe dysbiosis that is not restored to a healthy gut flora by a single dose of 50-80g FMT delivered to the cecum, or the cause of the problem is not in the gut. If additional functional comorbidity is a surrogate measure for somatization the results suggests that IBS with additional functional disorders is a dominating brain disorder, whereas IBS without is a disorder of the gut. As discussed in the introduction, a bidirectional

communication between the gut and the brain is suggested to be involved in IBS pathophysiology. IBS is a heterogeneous disorder with a diverse symptomatology. It is possible that a subgrouping based on the gut or the brain, as driver of the pathophysiology is possible, although very controversial. The effect of hypnotherapy in IBS also suggests involvement of brain-gut interactions in IBS. It would be very interesting to investigate if the subgroup with additional functional disorders benefits in particular from hypnotherapy, or other therapies with a top-down approach.

Self-reported additional functional comorbidity is not a validated, nor an adequate measure for somatization. It is now clear that a patient reported questionnaire that assess somatization tendency would have been interesting to include in paper 2 and 3, i.e. the Patient Health Questionnaire 15[131]. This would allow us to further explore if there were a subgroup with a low treatment response and increased somatization tendency.

IBS-subtype is a predictor of the treatment effect, but the effect did not point in a specific direction. An association between IBS subtype (IBS-D/M) and quality of life[132] nor between pain and IBS-subtype is neither found in other studies[133]. A specific signature in the microbiota that discriminate IBS-D from IBS-M is also not found[71]. However, to our knowledge, it is not assessed if the responsiveness to treatment by the IBS-SSS or IBS-QoL is IBS-subtype dependant.

Another possible reason for a subtype specific effect is that, one of four IBS-D patients may have bile acid malabsorption instead of (or in addition to) IBS, and the presence of this disorder may interfere with the treatment effect. The exclusion of bile acid mal absorption was not done according to the gold standard (SeHCAT scan). If there in the group with IBS-D was a mix of bile acid mal absorption and IBS, while the IBS-M group was only with IBS, it is possible that the effect of FMT was different between the two groups. However, secondary analysis with RM-ANOVA did not point in specific direction so the significance of not excluding bile acid mal absorption is uncertain. A possible way of assessing bile acid mal absorption in future studies (if SeHCAT scan is not available) is to do serum C4 tests. This is a simple accurate method for diagnosing bile acid mal absorption, but requires further clinical validation[134]. It would have been very interesting to see if a positive serum C4 test for bile acid malabsorption predicted the treatment response to FMT in the IBS-D subgroup.

IBS-like gastro-intestinal complaints is a part of the diagnostic criteria for chronic fatigue syndrome/myalgic encephalomyelitis[135]. More than 90% of IBS patients experience fatigue, whereas more than 90% of chronic fatigue syndrome/myalgic encephalitis patients experience IBS in their lifetime history[38,136]. Also a dysbiosis is suggested to be involved in chronic fatigue syndrome/myalgic encephalitis, as earlier mentioned. Thus, we expected an improvement in fatigue to be associated to excessive functional comorbidity. Instead we found self-reported depression before treatment to be the only additional predictor with a significant effect on the treatment response (paper 3). However, a recent study showed that in about half of cases, IBS symptoms are found to start first and psychological distress developing later, suggesting that mood disorders in IBS could be a gut disorder[6]. In participants with, compared to participants without self-reported depression, the baseline fatigue score was a two fold (paper 3 and table5). In addition self reported depression predicted a sustainable improvement in fatigue (figure 7A). Assuming that FIS score is a surrogate measure for depression, the finding suggests that depression in IBS is a gut disorder in a subset of IBS. A recent study found that administration of a probiotic altered brain activity, decreased depression score and the 4-creaol sulfate level[137]. Four-creaol sulfate is a substance produced from host-bacterial interactions that influences the dopamine/noradrenaline pathway in depression[71]. Retrospectively, a validated assessment tool for anxiety and depression should have been included in the study to assess the FMT effect on mood disorders.

Summarised, we found both gut-brain (self reported depression at baseline) and brain-gut interactions (self reported functional disorders other than IBS at baseline) to predict the treatment response. This suggests that a bidirectional communication between the gut and the brain is present in IBS pathophysiology. Self reported depression and excessive functional comorbidity at baseline was respectively positive and negatively associated to a treatment response. Our findings need to be confirmed with validated measures of somatization tendency and mood disorders. This is important because our findings suggest that patients with IBS and mood disorders or excessive functional comorbidity may represent subgroups with a different pathophysiology.

5.5 Limitations

There are several limitations to our study. Most are already discussed in this thesis and paper 1-3. Firstly, it is uncertain if our results from paper 1-3 are valid to patients diagnosed by the current Rome 4 criterion that was published after we started the study. However, the adjusted

Rome 3 criterion we applied is in very close resemblance to the new Rome 4 criterion. Second, one of the objectives was to find the prevalence of alternative disease in IBS (diagnosed by the Rome 3 criterion) by an extensive baseline assessment (paper 1). Although we found an interestingly high prevalence of MC in the age group over 50 years old, a test that could exclude bile acid mal absorption was not included in the baseline assessment. Based on other studies, it is likely that we would have found several cases. It would have been appropriate to offer these patients bile acid sequestrates before considering FMT. In addition, three of four cases with MC had already undertaken a colonoscopy before the study (paper 1). Although it is uncertain if pinch biopsies was obtained, this questions if one colonoscopy is enough to rule out MC. If this is the case it might be wrong to not include MC in a modified intention to treat analysis (paper 2 and 3). Third, we did not use the recommended adequate relief as the primary endpoint. Instead we used a global assessment tool (IBS-SSS) that mainly focuses on abdominal complaints in IBS (paper 2). Forth, fresh and frozen donor FMT was not randomized which precludes any firm conclusions regarding the effect of fresh vs. frozen donor FMT in IBS (paper 2 and 3). Fifth, we found variables associated to a lasting and sustainable FMT effect on both bowel related complaints, quality of life and fatigue (paper 2 and 3). However, the variables (i.e. self reported depression and absence of additional functional disorders) were not identified by validated questionnaires. Therefore, we can only suggest hypothesis of what the variables are surrogates for and what is their involvement in IBS pathophysiology. Sixth, our donor screening would not be acceptable by the current recommendations and standards[120,123]. The most obvious screening that is missing is testing the donors for multi resistant organisms. When the study was designed there were no guidelines (as far as we could find) for donor screening. However, this came to our awareness during the follow up of participants, and both donors tested negative for multi resistant organisms. There are still many open questions to how recruitment and screening of donors should be performed, but a thorough discussion of this topic is beyond the scope of this thesis. Sixth, studies of the microbiota are not included in paper 1-3 or this thesis. Thus, we cannot tell if the treatment response is associated to changes in the microbiota (paper 2 and 3). However, it is already established that in both Clostridium difficile infection and irritable bowel syndrome, phenotypic changes in the microbiota accompanies FMT[72,138,139].

The biggest concern regarding external validity, and a limitation in our results is that we have not assessed whether the effect seen in this study (paper 2 and 3) is specifically due to our donors' microbiome, or the mix of their individual microbiome. Only 14 of 664 genera

conform to a core microbiome in the gut[71]. Characteristics of super FMT donors exist, but are poorly assessed in IBS[140]. To our knowledge, a trial that randomise donors to assess if there is a donor specific effect has not been performed in IBS, nor in any other disease where the FMT effect is evaluated. The concept of FMT is to improve intestinal dysbiosis by transferring stool preparations containing a stable, viable, diverse, and normal community from a healthy donor. However, the therapeutic active agent(s) in FMT could just as well be elements of the virome, other components of the faecal water, or even products of the donor's human cells[122]. Efforts to assess the role of fungi and virus in IBS are sparse. One study found an association between visceral hypersensitivity and fungal dysbiois in IBS[141]. Presence of certain fungi can selectively inhibit repopulation of lactobacilli after a disruption and depletion. Conversely, bacteria (bacteroides) are found to promote colonization resistance to fungi. In addition, certain fungi and bacteria are pathogenic when combined in the gut flora, and not separately[142]. Studies of the virome in IBS could not be found. In inflammatory disease the virome is suggested to be a biomarker for disease, contribute to bacterial dysbiosis and a predictor for outcome of FMT therapy[143 144]. Like in IBS, FMT in inflammatory disease is an experimental treatment, but found effective in several studies[145]. Clostridium difficile infection is an intestinal dysbiosis with a known pathogen. FMT therapy in recurrent Clostridium difficile infection restores a functional healthy gut flora[146] and is found more effective than antibiotics[147]. A recent study found bacteriophage transfer during FMT in Clostridium difficile infection to be associated to the treatment outcome[148]. In addition, associations between alternations in fungal microbiota and disease flare is found in inflammatory bowel disease[149]. Thus, assessment of the microbiota and/or the microbiome would increase the scientific gain of this study, but there is currently no universal method that would give us a complete picture of all the possible players in the microbiome that may contribute to the IBS pathophysiology.

6 Conclusion

If IBS is approached as a positive diagnosis, clinicians should be aware of MC as a possible differential diagnosis, particularly in the subgroup of patients aged more than 50 with IBS-D. In this study we found a significant effect of FMT, when the active group was compared to placebo, on bowel related complaints by the IBS-SSS, quality of life by the IBS-QoL and fatigue by the FIS in the time course three to six months after treatment. The finding of a concurrent significant effect of FMT in favour of active treatment in each of the three patient reported outcome measures support the concept that IBS is closely related to the gut

microbiota and that FMT may have a role in the treatment of IBS. More in depth, the secondary analysis suggests that there are subgroups of IBS that differ, where more severe fatigue seems to be a gut disorder in the subgroup of IBS with concomitant depression, while abdominal complaints and poor quality of life seems to be a dominating central disturbance in the subgroup with functional disorders in addition to IBS. These findings are exploratory, but support the concept of a bidirectional communication between the gut and the brain, and the involvement of the microbiota-gut brain axis in IBS pathophysiology. FMT for IBS remains an experimental treatment also after this study. However, the findings strongly support initiating a phase three multi centre study to determine if FMT should be implemented into clinical practice for patients with IBS.

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8 List of appendices

Paper 1

Paper 2 including appendix

Paper 3 including appendix

IBS-diagnostic criteria and subtyping questionnaire

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Irritable Bowel Quality of Life questionnaire

Fatigue Impact Scale questionnaire

Five days dietary record

Extract of baseline self assessment questions from the baseline interviewer instructions

Consent form IBS participants

Consent form donors

Letter of approval REC North for project no 2013/971

Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial

Peter Holger Johnsen, Frank Hilpüsch, Jorunn Pauline Cavanagh, Ingrid Sande Leikanger, Caroline Kolstad, Per Christian Valle, Rasmus Goll

Summary

Background Irritable bowel syndrome (IBS) is a common condition characterised by abdominal pain, bloating, and poor quality of life. IBS might be caused by a gut dysbiosis. We aimed to compare faecal microbiota transplantation (FMT) with placebo in patients with IBS.

Methods In this double-blind, randomised, placebo-controlled, parallel-group, single-centre study, we enrolled patients with IBS with diarrhoea or with diarrhoea and constipation (excluding dominating constipation) defined by the ROME III criteria, scored as moderate to severe according to the IBS severity scoring system (IBS-SSS; a score of ≥175). Eligible participants were aged 18–75 years and were recruited locally by general practitioners in northern Norway. We randomly assigned participants (2:1) in blocks of six to active or placebo FMT. Personnel not involved in the clinical performance of the trial generated the randomisation sequence using a randomisation website. Non-study personnel performed the final allocation and standardised the active and placebo transplants to make them identical in appearance and temperature. The faeces were freshly processed, and were used the same day (fresh transplant) or were stored in a freezer for later use (frozen transplant); participants' own faeces served as placebo. A dose of 8 mg loperamide was administered orally 2 h before endoscopy to retain the transplant. The transplant (50–80 g of faeces mixed with 200 mL of isotonic saline and 50 mL of 85% glycerol) was administered by a colonoscope to the caecum. The primary endpoint was symptom relief of more than 75 points assessed by IBS-SSS, 3 months after FMT. The primary analysis was done in the modified intention-to-treat population, excluding participants who did not undergo treatment or who were diagnosed with any other disease by pinch biopsies obtained during the treatment procedure. For the safety analysis, only participants who did not undergo treatment were excluded. The study is registered with ClinicalTrials.gov, number NCT02154867. The trial has been extended with an open-labelled study treating the placebo group with frozen FMT for further exploratory studies.

Findings Between Jan 1, and Oct 30, 2015, we recruited 90 participants and randomly assigned them to active treatment (n=60) or placebo (n=30). Three participants did not undergo FMT and four were excluded after diagnosis of microscopic colitis, leaving 83 for final modified intention-to-treat analysis (55 in the active treatment group and 28 in the placebo group). 36 (65%) of 55 participants receiving active treatment versus 12 (43%) of 28 receiving the placebo showed response at 3 months (p=0·049). One participant had transient nausea and vertigo (active group) and was observed at the hospital for a few hours after the procedure. Two participants had soiling of transplant on their way home from treatment (one in each group) and three experienced self-limiting intermittent abdominal pain (one in the active group and two in the placebo group). No serious adverse events could be attributed to FMT.

Interpretation FMT induced significant symptom relief in patients with IBS. However, larger multicentre studies are needed to confirm the results.

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Introduction

Irritable bowel syndrome (IBS) is a functional gut disorder characterised by abdominal pain or discomfort associated with abnormal frequency and consistency of bowel movements. IBS presents as one of three phenotypes: IBS with diarrhoea, IBS with constipation, and IBS-mixed (ie, IBS with diarrhoea and constipation). IBS is a very common disease with a global prevalence of at least 11·2%.[1] The quality of life for patients with IBS is significantly impaired and the disease imposes a substantial economic burden on society by increased health-care expenditures.[2,3] Pharmacological treatment options are limited and dietary interventions can be cumbersome to maintain.[4,5] Alterations in the intestinal microbiota are gaining increasing interest as a cause for IBS and a dysbiosis of the gut flora is thought to be part of the pathophysiology of IBS.[6,7]

Faecal microbiota transplantation (FMT) has been suggested as a possible treatment option for restoring normal gut microbiota,[8–10] but no randomised trials have

Research in context

Evidence before this study

We searched PubMed for manuscripts published in English from inception and until Oct 20, 2015, with the terms "irritable bowel syndrome" in combination with "faecal transplantation", "faecal bacterio therapy", "randomized controlled trial", "dysbiosis", or "microbiota". We found no randomised controlled trials investigating the treatment effect of faecal transplantation in irritable bowel syndrome (IBS). Only a few uncontrolled small studies were found to report improvement. However, we found strong support for dysbiosis having a role in the pathophysiology in IBS and that a healthy microbiota could be restored by faecal microbiota transplantation (FMT).

Randomised controlled trials have shown relief in the symptom severity of IBS through manipulation of the microbiota or the substrates metabolised. Probiotics and antibiotics that target the microbiota have been shown to give relief of symptoms, as has a diet low in short-chained carbohydrates, which are fermented by the bacteria of the large intestine. Additionally, studies of microbiota composition show a less diverse microbiota in people with IBS than in healthy controls. Dysbiosis with a low diversity of the gut flora is reminiscent of alterations in the microflora in *Clostridium difficile* infection. FMT is superior to drugs for treating *C difficile* infection and a restoration of a healthy gut flora is seen after FMT treatment. The principle of normalisation of dysbiotic microbiota could be applied in IBS to induce symptom relief or potentially even remission of disease.

Added value of the study

We interpret our data as a proof of concept that the pathophysiology of IBS might be closely related to changes in the gut microbiota.

Implications of all the available evidence

In combination with the evidence from systematic reviews, the results of the trial show that FMT is feasible for patients with IBS, and presents as an affordable and effective treatment for a syndrome with limited treatment options. Our results will need to be confirmed in larger multicentre studies.

been done for such therapy in IBS.[9] The present study aimed to test the effectiveness of FMT in moderate-to-severe IBS.

Methods

Study design and participants

This was a double-blind, randomised, placebo-controlled, parallel-group, single-centre study done at the University Hospital of North Norway Harstad, Harstad, Norway, with 12 months of follow-up. The study was approved by the regional committee of medical ethics (REK-NORD: 2013/971).

Participants from the local area were made aware of the study by use of posters at doctors' offices and via the local news media. Local general practitioners initially screened participants according to the Rome III criteria. Eligible participants (aged 18–75 years) were then referred to Sjøkanten legesenter (a centre for general practice physicians), where they were reassessed with a questionnaire from the Rome Foundation for IBS with diarrhoea or mixed IBS according to the Rome III criteria.[11]

Participants with mixed IBS were only eligible if constipation was not the dominating symptom. To ensure responsiveness, participants were only eligible if they were scored as having moderate-to-severe IBS symptoms by the IBS severity scoring system (IBS-SSS; a score of ≥175),[12] and reported abdominal pain or discomfort at least once a week in the Rome Foundation questionnaire.[13,14] To avoid any potential adverse effects of the bowel preparation procedure, we did not include participants with severe cardiac disease, pulmonary disease, or kidney failure. To avoid other mimicking diseases, we did not include participants with nocturnal abdominal pain or long-lasting constant abdominal pain with no variability because these symptoms are atypical for IBS.[13] For safety reasons, we did not include participants with immune deficiency or if they used immuno-modulating medication. We also excluded participants who were assessed as likely to be non-compliant (ie, not adhering to the tasks they were to perform as participants).

We excluded participants if we found a reason to suspect an alternative diagnosis to the IBS complaints from our tests. Our investigations included physical examination, with special attention to the abdomen and lymph nodes; weight measurements (a body-mass index of less than 18 kg/m² led to exclusion); full medical history; and questions regarding the nature of experienced abdominal pain or discomfort (long lasting or nocturnal), red flags (blood in stool, weight loss, or night sweats), or food allergies. Additionally, we did blood tests, faecal tests, and pinch biopsies from the colon (at the time of FMT treatment) to exclude alternative diagnoses. Blood tests measured haemoglobin, complete blood-cell count, erythrocyte sedimentation rate, creatinine, sodium, potassium, C-reactive protein, aminotransferases, alkaline phosphatase, γ-glutamyltransferase, albumin, vitamin B_{12}, folic acid, ferritin, glycated haemoglobin, anti-tissue transglutaminase IgA, total IgA and IgE, thyroid-stimulating hormone, and thyroxine. Faecal samples were tested for occult blood, pancreatic elastase, calprotectin, and pathogenic bacteria (*Shigella* spp, *Salmonella* spp, *Campylobacter* spp, *Yersinia* spp, and toxin-producing *Clostridium difficile*). If participants had used any antibiotics in the period after assessment, we delayed treatment for 3 months and reassessed the participant before FMT. After assessment, patients

received information about the study and gave their written consent. Faecal samples and placebo faeces were obtained at the hospital, which is near to Sjøkanten legesenter (10 min walk). Most participants delivered the placebo samples after the screening at Sjøkanten legesenter 2–4 weeks before treatment at the hospital using the bathroom facilities at the hospital. We allowed participants to use the bathroom facilities at home as long as the transport time to the hospital was less than 2 h. When delivering from home, they used whatever container they preferred as long as it was clean.

Donors were recruited informally and screened at the hospital. We wanted to use as few donors as possible to standardise the transplant and decided to use two donors at first, but recruit more if they could not deliver in time or supply the quantities needed. Potential donors were first assessed by their medical history. Exclusion criteria included use of antibiotics in the past 3 months; new tattoos or piercings in the past 3 months; high-risk sexual behaviour; former imprisonment; or history of any of the following conditions: chronic diarrhoea, constipation, inflammatory bowel disease, IBS, colorectal polyps or cancer, immunosuppression, obesity, metabolic syndrome, atopic skin disease, or chronic fatigue. We then used faecal microscopy to screen for parasites, ova, and cysts; cultures for *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, *Yersinia* spp, and toxin-producing *C difficile*; faecal tests for *Helicobacter pylori* antigen, viruses (norovirus, rotavirus, Sapovirus, adenovirus), calprotectin, and occult blood; blood samples for glycated haemoglobin; and serology for HIV, *Treponema pallidum*, and hepatitis A, B, and C. Donors were screened before the first donation and when treatment of participants was finished approximately 7 months later. The donors provided faeces throughout the whole period that interventions were being done. We always made sure that at least four frozen transplants were ready in the freezer prior to a treatment day so that we could perform a full block of six (four frozen [active] transplants and two placebo).

Randomisation and masking

We planned to treat six consecutive participants per day. Originally, we intended to randomise in blocks of ten, but found it more practical to do a complete block each day of treatment. Thus, we changed the block size to six before we initiated treatment. A researcher, not involved in the trial, created the allocation sequence using a randomisation website. The treatment was randomised in fixed blocks of six (four active and two placebo) without stratification. This randomisation sequence was sealed in opaque envelopes, each corresponding to one treatment day. Each participant got a study number at enrolment depending on the first conveniently available slot for the participant to do the treatment.

A researcher placed placebo treatment in envelopes for all participants, and marked the envelope with the corresponding study number. An allocator (non-study personnel) then replaced placebo with active as determined by the block sequence. This was done in a closed room and all unused placebo transplants and the block allocation sequence were immediately disposed of. Envelopes with syringes were then placed in a refrigerator until use. The allocator also did the final temperature standardisation in the water bath. It was predetermined to perform the blocks with the fresh transplant as active treatment first, and frozen donor faeces as backup if the donors could not deliver fresh. To have the active treatment with fresh and frozen samples balanced, we switched to blocks of frozen when 30 fresh transplantations and 15 placebo transplantations had been performed. By then performing the remaining blocks with frozen to placebo in the ratio 2:1, we could do post-hoc analysis with fresh, frozen, and placebo in the ratio 1:1:1. Investigators were aware of the active to placebo ratio of 2:1 in each block and that the blocks with fresh transplant as active treatment were the first to be done. Patients only knew the 1:1:1 ratio of fresh to frozen to placebo. Otherwise, patients, investigators, and outcome assessors were kept masked to the allocation and intervention. The randomisation key was revealed to researchers when all participants completed the 12-month follow-up.

Procedures

All participants underwent FMT at the University Hospital of North Norway Harstad, Harstad, Norway, within 2–4 weeks of the assessment at the assessment centre.

Placebo and active transplants were prepared as follows: 50–80 g of freshly delivered faeces were mixed with 200 mL of isotonic saline and 50 mL of 85% glycerol, homogenised in a blender for 60 s, filtered through a 0·5 mm mesh steel strainer, drawn on 50 mL sterile Luer-lock syringes, and sealed.

Participants' own faeces served as placebo; placebo transplants from each participant were prepared 2–4 weeks before intervention and stored at –40°C. Frozen donor transplants were prepared and stored during the same period as placebo. Independent of whether fresh or frozen transplants were used as the active treatment in the block of six, the placebo transplants were thawed overnight at 5°C in a refrigerator, without transforming to liquid. If the block of six used frozen as active treatment, frozen donor faeces were thawed in the same way as placebo. If fresh samples were to be used as the active treatment in the block of six, the transplants were prepared 1 h before the first treatment in each block of six and refrigerated with the placebo until use. Thus, fresh transplants used late in a block were refrigerated for up to 6 h before use. 45 min before each administration, syringes (fresh, frozen, or placebo) were transferred from opaque envelopes in a refrigerator to a water bath (12°C) to ensure identical appearance and temperature.

For the **randomisation website** see www.randomization.com

Fresh donor samples were obtained up to 7 h before the FMT procedure. When preparing the donor transplant, we used a mixture from both donors, so that one donor could compensate for the other when small amounts of faeces were delivered. Both freshly prepared and premade frozen transplants were prepared using the same two donors.

No antibiotics were given before the procedure. The participants had a standard bowel lavage with sodium picosulphate plus magnesium citrate (Picoprep, Ferring) before intervention. A dose of 8 mg loperamide was administered orally 2 h before endoscopy to retain the transplant. The transplant was administered to the caecum through the biopsy channel of the endoscope after pinch biopsies for standard histology had been obtained. Participants in need of analgesics during endoscopy received 0·5–1·0 mg of intravenous alfentanil. After the intervention, the participants had no restrictions on activity level and were asked to keep an unchanged diet.

Participants received the self-assessment questionnaires (IBS-SSS, fatigue, and quality of life) by post at 2 weeks after treatment, and then at 1, 3, 6, and 12 months. A research nurse at Sjøkanten legesenter handled the logistics with the questionnaires.

To monitor any changes in dietary intake, particularly the intake of FODMAPs (ie, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols), when being screened at Sjøkanten legesenter, participants were given thorough instructions compiled by a registered dietitian on how to complete a 5-day dietary record at baseline and after 3 months. Participants were asked to register all intakes of food and beverages, including probiotics. To limit the workload on participants, we did not obtain a dietary record at 12 months. We analysed dietary records using the software Dietitian Net Pro (Diet and Nutrition Data, Bromma, Sweden), consisting of the Norwegian Food Composition table,[15] supplemented by available published values of FODMAPs[16–18] and analysis of a selection of Norwegian bread (Gry Skodje, University of Oslo, Oslo, Norway; personal communication). At Sjøkanten legesenter, all participants reported a complete list of all medications taken before receiving FMT and at 12 months after receiving FMT to detect any changes in medications that could affect the outcome measure. We systematically reviewed these lists, in particular looking for changes that could affect symptom intensity; in the active group, we only counted changes in medication that might give symptom relief, and in the placebo group, we counted changes that might give symptom aggravation.

In a baseline non-validated self-assessment questionnaire, we mapped the potential confounding variables (fibromyalgia, chronic fatigue syndrome, jaw pain, pelvic pain, anxiety, and depression); all known to be associated with IBS functional comorbidity. These are factors known to affect both symptom severity and responsiveness to interventions in clinical trials.[14] We did not do additional investigations to verify if the participants fulfilled the diagnostic criteria for these self-reported confounders. Additionally, we asked if the participants could relate the IBS to a triggering cause. To account for use of antibiotics that could affect the engraftment process, and thereby the outcome, we asked if antibiotics were used during the follow-up period when each participant completed the trial.

We also collected patient-reported outcome questionnaires to assess the effect of FMT on fatigue (assessed before FMT and at 3, 6, and 12 months after FMT) and quality of life (assessed before FMT and at 6 and 12 months after FMT); the results for these will be reported elsewhere.

Outcomes

The primary endpoint was symptom relief of more than 75 points assessed by IBS-SSS, 3 months after FMT. This was reassessed at 12 months after FMT for the secondary endpoint. Translation of the IBS-SSS from English to Norwegian was done in cooperation with, according to procedures set out by, and under guidance from the Rome Foundation. The IBS-SSS evaluates retrospectively the intensity of IBS symptoms during the past 10 days: abdominal pain, distension, stool frequency and consistency, and interference with life in general. Each item is scored on a visual analogue scale from 0 to 100 and the score for all five summed. A total score of 175–300 is deemed moderate severity and a score of more than 300 is deemed severe.

Statistical analysis

Results from case reports and an open-labelled study[8] were used to calculate the sample size for this trial. The placebo effect in IBS is known to be high, but the effect might be less than 20% for trials running for 3 months or longer.[19] Higher placebo response is associated with longer duration of treatment and a greater number of office visits.[14] In this trial, there was only one treatment procedure, and no office visits during follow-up. Consequently, we estimated a placebo effect of 10%. Estimating that response rates of 50% in the active group would be clinically relevant, we calculated that we would need 50 participants in a balanced two-group design (α=0·05; 1–β=0·80). To allow for dropouts, we initially planned to enrol 60 participants.

However, to account for logistical difficulties relating to use of fresh donor faeces, we altered the protocol (after registration at ClinicalTrials.gov, but before enrolment of participants) to add a further 30 participants to the active group to allow for the use of frozen donor faeces, thus avoiding having a block of six participants prepped for colonoscopy with no active transplant available if donors were not able to deliver fresh faeces. Of note, the study was not designed or powered to compare outcomes between fresh and frozen transplants in the present study protocol, but we planned to compare fresh and frozen

transplants separately with placebo in a post-hoc analysis. Additionally, we knew that the expected placebo effect at 3 months might be an underestimate. As such, we did not change our power calculation.

To maintain blinding, an independent research group from the University of Oslo did an interim analysis of efficacy and safety after the first 67 participants had passed the 6-month registration and found no reason to terminate the study protocol due to serious adverse events or symptom aggravation in the active group.

For the primary and secondary endpoints, we compared the proportion of participants who responded to active treatment with the proportion who responded to placebo, with a responder defined as a participant with more than a 75 point decrease in IBS-SSS. This endpoint was analysed in cross tables using the χ^2 test. Post hoc, we investigated the effect of fresh and frozen transplants versus placebo in the recorded timecourse of IBS-SSS scores in a repeated measures analysis of variance (RM-ANOVA) and explored other variables that might predict treatment effect, such as additional functional comorbidity and IBS subtype. We also tested for potential confounding. Each of the following variables was entered into the model one at a time to test stability of the fit (and removed again if no effects were detected): sex, age, psychiatric comorbidity (self-reported anxiety and depression from the baseline questionnaire), antibiotics during the study, use of loperamide during the study, and change in FODMAP intake based on dietary records.

The European Medicines Agency and the US Food and Drug Administration suggest that trials of interventions for IBS should use a primary endpoint of abdominal pain intensity, with stool frequency assessed as a secondary endpoint.[20,21] This was not planned for in the protocol; however, post hoc, we have broken down the IBS-SSS into the five individual components in a doubly multivariate RM-ANOVA. These components include pain intensity, but not stool frequency.

All statistical analyses of efficacy were based on a modified intention-to-treat population, excluding participants who did not undergo treatment and participants diagnosed with any other disease by the pinch biopsies obtained during the treatment procedure. For the safety analysis, only participants who did not undergo treatment were excluded. The analyses were done using IBM SPSS 24.0 software. This study is registered with ClinicalTrials.gov, number NCT02154867. The trial has been extended with an open-labelled study treating the placebo group with frozen FMT for further exploratory studies.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

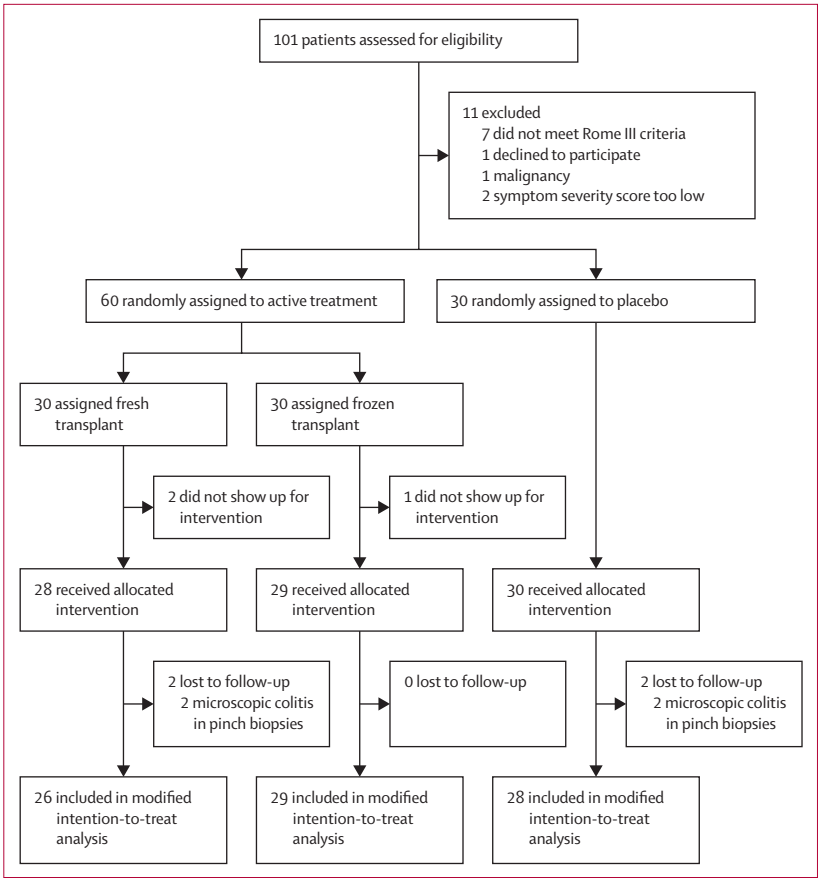

Figure 1: **Trial profile**

Results

Between Jan 1, and Oct 30, 2015, we assessed 101 individuals for eligibility, of whom 11 were not eligible (figure 1). Of the 90 participants randomly assigned to a treatment group, we excluded four after the FMT procedure because a diagnosis of microscopic colitis emerged after histology.[22] We referred these four to their primary physician to receive the appropriate treatment for this condition. Three of the randomly assigned participants did not show up on the day of the FMT procedure. This left 83 participants for the final modified intention-to-treat analysis (figure 1).

Baseline demographics and clinical characteristics were similar in the two study groups (table 1 and appendix p 1). The FODMAP intake registered in the dietary records did not change significantly between baseline and 3 month follow-up in either group (median difference 0·0 g/day [IQR –6·9 to 4·9] in the active group; 0·0 g/day [–4·0 to 4·7] in the placebo group). 51 participants could not identify a triggering event for their IBS, thus we could not analyse whether the effect of FMT might differ by triggering event. In the dietary records, only two participants reported intake of a probiotic supplement in the 3 months after FMT.

The assessment of prescribed medications revealed that, between baseline and 12 month follow-up, one

See Online for appendix

participant receiving frozen FMT was prescribed a tricyclic antidepressant and had symptom relief. Additionally, there were three other cases of change in medication that might be relevant, but for the three cases the IBS score did not change in favour of a positive result. One participant in the active group was prescribed loperamide 2 mg three times a day, but did not show any symptom improvement. Another participant, in the placebo group, was prescribed metformin 500 mg twice a day; however, the participant had a major improvement in severity score at 12 months. A third participant, in the placebo group, discontinued loperamide during the trial without any change in severity score.

36 (65%) of 55 participants receiving active treatment versus 12 (43%) of 28 receiving the placebo showed a response of a decrease in IBS-SSS of more than 75 points at 3 months after FMT (p=0·049); 31 (56%) of 55 participants receiving active treatment versus ten (36%) of 28 receiving placebo had a similar degree of response at 12 months after FMT (p=0·075).

A post-hoc analysis of IBS-SSS scores during the whole study, using a repeated measures ANOVA with treatment group, IBS subtype, and presence of other functional disorders as predictors in a full-factorial model, optimised by removing non-significant terms, showed that, of the potential confounding factors, none of the variables examined (sex, age, psychiatric comorbidity, antibiotics, use of loperamide, or change in FODMAP intake) had a significant effect by itself (appendix p 2).

A further post-hoc analysis comparing participants who received fresh transplants, those who received frozen transplants, and those who received placebo, unadjusted for other functional comorbidities, suggested that participants who received frozen FMT had lower IBS-SSS scores throughout follow-up than did those who received fresh FMT despite a higher mean baseline, and that participants who received fresh FMT showed no response compared with those in the placebo (figure 2A). A large and long-lasting placebo effect was also apparent (figure 2A). However, after adjustment for other functional comorbidity, both active FMT formulations had a similar effect on IBS-SSS scores (figure 2B).

Finally, we did a post-hoc analysis of the individual components of the IBS-SSS using the same predictors as in the RM-ANOVA analysis in a doubly multivariate RM-ANOVA. The two main complaints of IBS, pain and bloating, in addition to reports of being content with bowel habits, were the most important contributors to the detected signal (appendix p 3).

One serious adverse event was reported: a participant in the active group was admitted to hospital for a few hours of observation after the FMT procedure due to transient vertigo and nausea (table 2). Other adverse events are reported in table 2. We deemed this to be related to the medication and instrumentation used during colonoscopy. None of the participants reported fever after FMT. None of the participants reported any new diagnosis or lasting side-effects 1 year after FMT.

	Placebo (n=28)	Active (n=55)
Age (years)	45 (34 to 57)	44 (33 to 54)
Sex		
Women	19 (68%)	36 (65%)
Men	9 (32%)	19 (35%)
IBS subtype		
IBS with diarrhoea and constipation (mixed)	15 (54%)	24 (44%)
IBS with diarrhoea only	13 (46%)	31 (56%)
Time with IBS (years)	10 (6 to 16)	10 (5 to 19)
IBS-SSS at inclusion	278 (223 to 254)	260 (226 to 313)
Functional disorder comorbidity*	9 (32%)	14 (25%)
Total FODMAP before FMT (g/day)†	0·0 (–4·0 to 4·7)	0·0 (–6·9 to 4·9)

Data are median (IQR) or n (%). FODMAP=fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. FMT=faecal microbiota transplantation. *Self-reported by questionnaires at inclusion; includes fibromyalgia, chronic fatigue syndrome, jaw pain, and pelvic pain. †Calculated from the 5-day dietary record. IBS=irritable bowel symptom. SSS=severity scoring system.

Table 1: **Baseline characteristics**

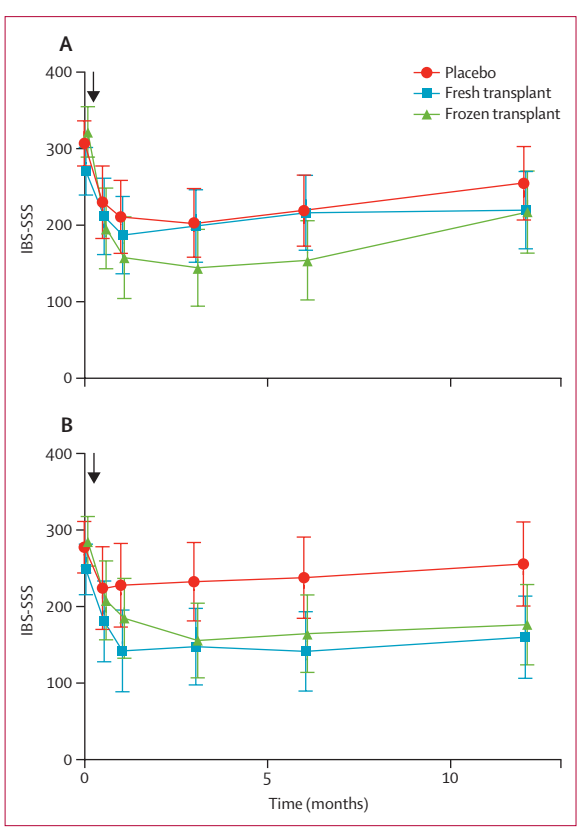

Figure 2: **Post-hoc analysis of the effect of FMT in IBS**
Arrow indicates intervention. Data are estimated marginal mean (95% CI) IBS-SSS from a three-way RM-ANOVA. (A) Unadjusted IBS-SSS scores. (B) IBS-SSS scores adjusted for other somatic functional comorbidity (time × group × functional). IBS=irritable bowel symptom. SSS=severity scoring system.

	Active (n=57)	Placebo (n=30)
Soling of transplant	1	1
Self-limiting intermittent abdominal pain	1	2
Self-limiting nausea and vertigo	1	0

Data are n.

Table 2: **Adverse events**

Discussion

To the best of our knowledge, this is the first randomised, double-blind, placebo-controlled trial of FMT for IBS. Our results show a significant effect of active treatment on IBS-SSS after 3 months but not at 12 months. In a post-hoc analysis adjusting for associated functional disorder comorbidity, we found similar effects for both fresh and frozen FMT formulations (figure 2).

Previous findings of symptom relief from a diet low in FODMAPs, which are fermented by colonic microbiota,[4] suggest a functional pathogenic effect of the dysbiotic microbiota.[23] The present data support this hypothesis and further suggest that FMT might be a way to correct this underlying cause of IBS. However, any conclusions to this end must await in-depth analysis of the gut microbiota after FMT. Alternative explanations to the positive effect of FMT, such as change in diet to low FODMAP and use of antibiotics, did not show any effects when we examined potential confounding factors in an RM-ANOVA analysis. Moreover, we observed no differences in medications or use of probiotics that could account for our findings.

A transplant seems to have a functional output within the first days after administration,[24] but engraftment of transferred microbiota can take at least 7 months after FMT,[25] and exogenous and endogenous factors could disturb this complex process.[26] These factors could explain why some participants relapsed in severity score in the 3–12-month period. When treating *C difficile* with FMT, the proportion of patients cured has been shown to increase with repeated treatment using an alternative donor.[27] Repeated treatment for participants who relapse, and for non-responders, with FMT from a different donor might yield similar results in IBS. Theoretically, an increased retention time would facilitate the engraftment process. However, we did not keep record of retention time. On the basis of the laxative effect of glycerol in all transplants, and that a few participants had soiling on their way home, we estimate that participants kept the transplant for 30 min to 2 h. Optimisation of the transplant formulation to minimise the glycerol content would probably help increase retention time.

By using RM-ANOVA, we were able to adjust for additional factors affecting symptom scores and potential confounders, allowing us to adjust for important covariates known to affect symptom severity. Figure 2 clearly shows the complexity in the assessment of severity in IBS by showing that other functional comorbidities (eg, fibromyalgia, chronic fatigue syndrome, jaw pain, and pelvic pain) have to be addressed when assessing response to treatment. Additional self-reported functional conditions might be negative predictors of treatment effect. Whitehead and colleagues[28] suggest that the prevalence of other functional diseases in individuals with IBS is not that different from the general population; however, a subgroup of patients with IBS also has a somatisation tendency. Excessive general somatic symptoms might be markers for somatisation and predominantly psychological IBS aetiology,[29] which is less likely to respond to FMT treatment. This signal might be what is detected in the model. After adjustment for self-reported comorbid functional disease, the effect of FMT with fresh and frozen samples seemed to be the same.

During the 87 FMTs performed, one serious adverse event occurred, which was probably related to the endoscopy. Generally, patients find the concept of FMT intriguing and we had no problems recruiting participants for the trial. As such, we find the procedure safe and feasible in this patient group with otherwise limited treatment options.

This study has four main strengths. First, participants were recruited in general practice and the sample is thus likely to be representative of the average patient with IBS. Second, we assessed potential participants with a validated questionnaire, which is especially important in a functional disorder without objective defining criteria. Third, the baseline descriptive parameters in table 1 show that the groups are fairly balanced, and very close to what is found in epidemiological studies.[30] Fourth, the long follow-up is important to compensate for the well-known high placebo effect in this patient group.

However, the study also has a number of weaknesses. First, because of insufficient funding, we could not support the clinical assessments with microbiota analysis, and therefore cannot associate engraftment of the transplant directly to symptom improvement. Second, the demonstrated effect might be donor dependent. However, by mixing faeces from two donors, we could not investigate whether one donor yielded a better response than the other. Third, because of the logistics of active transplants, we did not randomly assign freshly prepared and frozen FMT separately and thus cannot compare differences in effectiveness between them. Fourth, fresh transplant for participants late in a block of six was refrigerated for up to 7 h, which might have affected the microbiota composition. Fifth, we did not do a run-in period as recommended in guidance for IBS trials.[14] A run-in period would allow exclusion of participants with great symptom variability, thus reducing the noise in the dataset. However, the aim of our study was to test the effect of FMT in a general IBS population with moderate-to-severe symptom burden to get a result that reflects real-life practice. Sixth, our

primary outcome is not in line with the recommendations provided by the European Medicines Agency and the US Food and Drug Administration, which suggest the use of abdominal pain intensity as the primary endpoint and assessing stool frequency as a secondary endpoint.[20,21] The value of stool frequency in this context is debatable.[12] Moreover, these measures only reflect a subset of the Rome III criteria, which were used in the trial. If we had adhered to these recommendations, our assessment of discomfort would have been suboptimal; as such, we chose to use the global score of the IBS-SSS as the endpoint. Our post-hoc analysis showed that FMT has an effect on two of the main complaints in IBS—ie, pain intensity and bloating. Lastly, our initial power calculation used a low placebo response of 10%. In hindsight, this was unrealistic and the observed statistical power of the study is therefore somewhat lower than we estimated.

In conclusion, to the best of our knowledge, we have done the first randomised controlled trial on the effects of FMT in IBS. The outcome showed a significant symptom improvement in the active treatment group at 3 months, although this was not maintained at 12 months. Our results support the concept that IBS pathophysiology is closely related to the gut microbiota. However, the findings need to be confirmed in larger studies.

Contributors

PHJ, RG, FH, ISL, JPC, and PCV contributed to the study design. PHJ, FH, CK, and PCV collected the data. RG, PHJ, and CK did the data analysis and made the figures. RG, PHJ, and ISL interpreted the data. PHJ, RG, JPC, ISL, and FH prepared the manuscript.

Declaration of interests

We declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Johnsen PH, Hilpüsch F, Cavanagh JP, Leikanger IS, Kolstad C, Valle PC, Goll R. Faecal microbiota transplantation versus placebo for moderate-to-severe irritable bowel syndrome: a double-blind, randomised, placebo-controlled, parallel-group, single-centre trial. *Lancet Gastroenterol Hepatol* 2017; published online Oct 31. http://dx.doi.org/10.1016/http://dx.doi.org/10.1016/S2468-1253(17)30338-2.

Appendix Table I: Baseline characteristics

	Fresh (26)	Frozen (29)	Placebo (28)
Age median [range]	44 [22 68]	43 [19 70]	45 [24 71]
Female	69 %	62 %	68 %
IBS subtype (M/D)	46 %/54 %	41 %/59 %	54 %/46 %
Years with IBS (median[range])	10 [2 68]	10 [1.5 40]	10 [1 46]
IBS-SSS at inclusion (median[IQR])	249 [56]	283 [106]	279 [120]
Functional disorder comorbidity*	27 %	24 %	32 %
Total FODMAP before FMT g/day (median [IQR])#	10.5 [17.6]	10.6 [12.0]	10.0 [16.7]

Baseline characteristics and potential follow-up confounders. Values are median (95% CI) unless otherwise stated. Self-reported functional disorder comorbidity includes fibromyalgia, chronic fatigue syndrome, jaw pain, and pelvic pain. No significant differences in baseline characteristics were detected between groups (by Chi-square, oneway-ANOVA, or Kruskal-Wallis ANOVA as appropriate). *Self reported by questionnaires at inclusion; # Calculated from the five-day dietary record.

Appendix Table II: RM-ANOVA model

Within subjects effects	**df**	**F**	**P**
Time	4.92	26.8	< 0.0005
Time * treatment group	9.8	2.0	0.034
Time * other functional disorders	4.9	2.6	0.027
Time * treatment group * other functional disorders	9.8	2.5	0.007
Time * treatment group * IBS subtype	14.8	2.3	0.004
Time * treatment group * other functional disorders * IBS subtype	14.8	1.9	0.026
Between subjects effects			
Treatment group	2	1.09	0.340
Other functional disorders	1	1.95	0.166
Treatment group * other functional disorders	2	2.37	0.101
Treatment group * IBS subtype	3	0.63	0.596
Treatment group * other functional disorders * IBS subtype	3	0.40	0.751

The final repeated measures ANOVA model where non-significant terms (time * IBS subtype and time * IBS subtype * other functional disorders) are removed. All within subject effects corrected for deviation from sphericity by the Huyhn-Feldt method (Mauchlys W 0.542 df 14; P<0.0005; epsilon 0.984)

Appendix Table III. Doubly multivariate RM-ANOVA

Term	Q1 Abdominal pain VAS	Q2 Abdominal pain frequency	Q3 Bloating VAS	Q4 Content with bowel habits VAS	Q5 Impact on quality of life VAS
Time	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**	**<0.0005**
Time*treatment group	**0.034**	0.179	**0.007**	0.858	0.152
Time*other functional disorder	0.563	0.253	**0.001**	0.113	0.419
Time*treatment group*IBS subtype	**0.029**	0.067	**0.004**	**0.010**	0.055
Time*treatment group*other functional disorder	**0.004**	0.103	**0.012**	**0.038**	0.055
Time*IBS subtype*other functional disorder	0.950	0.277	**0.030**	0.675	0.657
Time*treatment group*IBS subtype*other functional disorder	0.266	**0.012**	**0.005**	0.244	0.167

A doubly multivariate repeated-measures ANOVA of all five questions in IBS-SSS based on the same predictors as the analysis of the total score. P-values corrected by the Huynh-Feldt method for all five questions.

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The effect of fecal microbiota transplantation on IBS related quality of life and fatigue in moderate to severe non-constipated irritable bowel: Secondary endpoints of a double blind, randomized, placebo-controlled trial

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ABSTRACT

Background: Severity in irritable bowel syndrome (IBS) is associated to impaired quality of life and fatigue. Fecal microbiota transplantation (FMT) induces significant relief in gastro-intestinal related complaints. The objective was to evaluate the effect of FMT on the secondary endpoints: IBS-related quality of life and fatigue in patients with non-constipated IBS.

Method: In this double-blind randomized placebo-controlled, parallel-group, single-center study, we enrolled patients with non-constipated IBS, defined by the ROME 3 criteria. We randomly assigned participants (2:1) in blocks of six to active or placebo FMT. Responder in fatigue and quality of life were defined as a decrease of 20 points in total Fatigue Impact Scale score, and improvement of 14 points in the IBS-quality of life questionnaire, respectively. In a modified-intention-to-treat population, we excluded participants who did not undergo treatment or who were diagnosed with any other disease by pinch biopsies during the treatment procedure.

Findings: Between Jan1, and Oct 30, 2015, we recruited 90 participants and randomly assigned them to active treatment (n = 60) or placebo (n = 30). Three participants did not undergo FMT and four were excluded after diagnosis of microscopic colitis, leaving 83 for final modified intention-to-treat analysis (55 in the active treatment group and 28 in the placebo group). Significant improvement in QoL (Odds ratio (OR) 3,801; confidence interval (CI) = 1,309−11,042 p = 0.011) and fatigue (OR = 4,398; CI = 1,175−16,468 and p = 0,020) was found at six months. Absence of other self reported functional disorders and presence of depression at baseline is suggested to predict a lasting effect of FMT in QoL and fatigue, respectively.

Interpretation: FMT induced significant relief in quality of life and fatigue. Results suggest a lasting effect of FMT in subgroups that should be further investigated in future studies. Funding Helse Nord, Norway and the Norwegian Centre of Rural Medicine, University of Tromsø, Norway.

1. Introduction

Irritable bowel syndrome (IBS) is a functional gut disorder characterized by abdominal pain related to abnormal frequency and consistency of bowel movements. In clinical practice and studies, participants are often categorized by the phenotypes: IBS with diarrhea, IBS with constipation and mixed IBS (i.e., IBS with alternating diarrhea and constipation).

IBS is associated with substantial costs to patients, healthcare system and society in terms of increased health care expenditures, loss of work productivity and decrease in quality of life (QoL) [1−3]. Patients are found willing to give up 10−15 years of their life expectancy for an immediate cure [2]. The severity of IBS is correlated negatively with QoL and positively with healthcare seeking [4]. We recently published the main results from the REFIT study, a double blind placebo-controlled trial on fecal microbiota transplantation (FMT) in moderate to severe non-constipated IBS. We found a beneficial effect on gastro-intestinal related complaints with number needed to treat of five [5].

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Research in context

Evidence before this study

We searched PubMed for manuscripts published in English from inception and until Sept 20,2019, with the terms "irritable bowel" in combination with "fecal transplantation", "fecal bacterio therapy", "randomized controlled trial", "dysbiosis", "quality of life", "fatigue", or "microbiota". We identified five randomized controlled trials assessing the effect of fecal microbiota transplantation (FMT) in irritable bowel syndrome (IBS) with diverging results. Among others, differences in route and number of FMT administration, outcome measures, processing of transplants and criteria for inclusion of donors and FMT recipients can explain the lack of consistency in the results. Only one RCT with a single colonoscopic administration of FMT was identified (Holster et al. 2019, Clinical and Translational Gastroenterology). This study found a significant effect of FMT on IBS related quality of life in the donor FMT group, but not in the placebo group. There was not a significant difference between groups, but the study included only 17 patients. We could not find any studies assessing the effect of FMT on fatigue in IBS.

Added value of this study

The data show that FMT may improve quality of life and fatigue in IBS, in particular in the subgroups with no excessive functional comorbidity and self reported depression, respectively. The study also highlight fatigue as a part of IBS symptomatology, and available for therapeutic interventions.

Implications of all the available evidence

The study, combined with our previous reported result, show that there is a consistent effect of FMT in bowel related complaints, quality of life and fatigue in IBS. In future studies an effort should be made to determine which IBS subgroups benefit from FMT treatment.

The European Medicines Agency recommends assessing the treatment effect by abdominal pain/discomfort along with abnormalities in defecation [6]. However, the symptom burden in IBS is diverse and extends beyond these gastro-intestinal complaints [4]. Fatigue is experienced in ninety percent of IBS patients, one of the most pronounced domains with decrements in health related QoL, and is moreover found as an independent predictor for referral to the secondary health care [1,7].

In this analysis of secondary endpoints from the REFIT study we aim of to evaluate the effect of FMT on IBS-related QoL and fatigue.

2. Methods

2.1. Study design and patient population

This was the secondary endpoints of a randomized, double blind, placebo-controlled, parallel group, single-center trial (NCT02154867) and the patient population and study design has been previously described [5]. Briefly, individuals between 18–75 years of age with a diagnosis of non-constipated IBS (based on Rome 3 criteria) with moderate to severe IBS by the IBS-SSS (cut-off 175 IBS-SSS score) were eligible for the study.

Participants were randomized in blocks of six for active or placebo FMT (4:2). Non-study personnel generated the randomization sequence using a randomization website. Placebo and active transplants were prepared by the same procedure and standardized to be identical in appearance and temperature at assigning of treatment. Placebo was participants' own feces obtained, processed and frozen during the inclusion assessment. Active treatment was processed donor feces. If frozen; processed and frozen 2–4 weeks before treatment and thawed at the day for allocation and treatment assignment. If fresh; collected and processed the same day as allocation and treatment assignment. It was predetermined if the active treatment in each block was fresh or frozen. We balanced the use of fresh and frozen active transplants to a ratio of 1:1. Transplants was made of 50–80 g of faces homogenized in 200 mL of isotonic saline and 50 mL of 85% glycerol and filtered.

After a bowel lavage, all participants underwent FMT at the University Hospital of North Norway, Harstad, within 2–4 weeks after the initial assessment. Treatment was delivered through the working channel of a colonoscope to the cecum. Participants, investigators and outcome assessors were kept blind to the allocation and intervention.

2.2. Outcomes

Secondary outcomes were to evaluate the effect of donor FMT vs. autologous FMT on fatigue (by the fatigue impact scale) at three six and twelve months and quality of life (by the irritable bowel quality of life) at six and twelve months.

2.2.1. The fatigue impact scale (FIS)

FIS is a 40 item questionnaire that assess the individuals' attribution of functional limitations to their subjective experience of fatigue in an overall score with three subdomains (cognitive, physical and social fatigue) [8]. Each item is scored on a 5-point Likert response scale (0 = "no problem" 1 = "small problem" 2 = "moderate problem", 3 = "big problem" 4 = "extreme problem"). Higher scores indicate increased level of subjective experienced fatigue (range, minimum score 0 and maximum score 160). The Norwegian version of FIS is validated for use in IBS [8]. A conservative measure of minimal clinical important difference, validated in patients with multiple sclerosis, is a decrease of 20 in total score [9]. FIS was administrated 2–4 weeks before treatment, and at three, six and twelve months after.

2.2.2. IBS-Quality of life questionnaire (IBS-QoL)

Quality of life was assessed using a validated 34-item questionnaire (IBS-QoL) with seven subdomains (dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual and relationships). Each item is scored on a 5-point Likert response scale (1 = "not at all" 2 = "slightly" 3 = "moderately" 4 = "quite a bit" and 5 = extremely"). Data were transformed to a sum score (range, minimum score 0 and maximum score 100). Transformation involved reversing all scores so that higher score indicated higher QoL, and then subtracting the lowest possible raw score from the actual raw score, dividing by possible raw score range, and multiplying by 100 [10]. IBS-QoL was administrated 2–4 weeks before treatment, and at six and twelve months after treatment. Minimally clinical important difference is an improvement of 14 in total IBS-QoL transformed score [11].

2.2.3. Metadata

In a self-assessment questionnaire before FMT treatment, we asked patients to report disorders that are associated to the severity in IBS [4]. This included other functional disorders (fibromyalgia, chronic fatigue syndrome, jaw and pelvic pain syndromes) and mood disorders (anxiety and depression). To assess the effect of diet on the results including the intake of FODMAPS (i.e., fermentable oligosaccharides, disaccharides, monosaccharaides and polyols) and probiotics, participants registered a 5-day prospective dietary record at baseline and after 3 months. The records were analyzed using the software Dietician Net Pro (Diet and Nutrition Data, Bromma, Sweden) [5]. In addition, all participants reported a complete list of

medications pre FMT and at 12 months post FMT to detect any changes in medications that could affect the outcome measure. Participants were also asked to report any use of antibiotics during the follow up period from baseline and until 12 months after treatment. We also assessed intestinal complaints by the irritable bowel symptom severity score (IBS-SSS) before treatment and at 1, 3, 6 and 12 months. Those results have been reported previously [5].

2.3. Statistical analysis

The statistical analysis plan for the secondary endpoints was not adequately elaborated in the study protocol as we intended to use the same statistical analysis for the secondary as we did for the primary endpoints previously reported in the Lancet Gastroenterology and Hepatology [5]. In the secondary endpoints (as in the primary endpoint) we compared the proportion of participants who responded to active treatment with the proportion of participants who responded to placebo by cross tabs using Chi Square. A responder in IBS-Qol was defined as an improvement in total score of 14 or more, and a responder in FIS was defined as a decrease of 20 or more in total FIS score. Since fresh and frozen donor feces were randomized to placebo as one active group it was not appropriate to compare fresh vs. frozen vs. placebo in the primary analysis.

In a post hoc analysis of IBS QoL, we compared the effect between placebo, fresh and frozen donor FMT in the recorded time course in a repeated measures analysis of variance (RM-ANOVA) and explored other factors that might predict the treatment effect. These included IBS-subtype, functional comorbidity (from the self-assessment questionnaire at baseline). We also tested for potential confounding. Each of the following variables was entered into the model one at a time to test stability of the fit (and removed again if no effects were detected: sex, age, antibiotics during study, use of loperamide during study, change in FODMAP intake based on dietary records, and mood disorders (anxiety and depression)). Finally, we did a doubly multivariate RM-ANOVA on the subdomains of the QoL to assess the significance of each subdomain to the change in total score.

In a post hoc analysis of FIS we first did a RM-ANOVA with the same factors associated to a treatment effect in IBS-QoL (fresh, frozen and placebo, IBS-subtype and functional comorbidity). No significant effects were found. We then did a RM-ANOVA with treatment group (fresh, frozen and placebo) and depression (from the self-assessment questionnaire at baseline) as predictors, and found one significant term. In the confounder control each of the following variables was entered into the model one at a time to test stability of the fit (and removed again if no effects were detected: sex, age, antibiotics during study, use of loperamide during study, and change in FODMAP intake based on dietary records, functional comorbidity and IBS-subtype). When stability of the fit was established, fresh and frozen was combined in to one active group in a new RM-ANOVA with active vs. placebo and depression as predictors. Once again terms not significant was removed and the confounder control was repeated with the same confounders as above. Finally, we did a doubly multivariate RM-ANOVA on the subdomains of the FIS to assess the significance of each subdomain on the total score. All data were analyzed using SPSS, version 25 (SPSS Inc, Chicago, IL, USA).

3. Results

One-hundred-and-one individuals from primary care were assessed for eligibility (between Jan1, and Oct 30, 2015); of those, 90 were included and 83 remained for the final analysis; four were excluded after being diagnosed with microscopic colitis after the intervention colonoscopy and three did not show up on the day of intervention (Fig. 1) [5]. Baseline demographics and disease characteristics are found in Table 1 (earlier reported in the Lancet Gastroenterology and Hepatology 2018). All baseline demographics were similar between groups.

There were no significant differences in baseline overall and subdomain score between groups in FIS and QoL-score except for the subdomain dysphoria in the QoL questionnaire (active 58,9 ± 23,8 vs. placebo 47,5 ± 25,0 p = 0,046). Mean scores with standards deviations and p-values for the difference between groups by independent sample t-tests are found in appendix, Table 1.

A significantly greater proportion of patients in the active treatment group achieved minimal clinically important improvement in the IBS QoL score from baseline to 6 months compared to the placebo group, (85% vs. 61%, Odds ratio (OR) 3801; confidence interval (CI) = 1309−11,042 and p = 0,011;). The corresponding difference at 12 months was not significant (78% vs. 61%, OR = 2319; CI = 0,860−6254 and p = 0,093;). A significantly greater proportion of patients in the active treatment group achieved minimal clinically important difference in the FIS score from baseline to 6 months (active treatment 35% and placebo 11%, OR = 4398; CI = 1175−16,468 and p = 0,020) but not at 3 months (active treatment 31% and placebo 18%, OR = 2058; CI = 0,669−6330 and p = 0,203) nor at 12 months (donor treatment 31% and placebo 32%, OR = 0,944; CI = 0,355−2511 and p = 0,909;).

In a post-hoc RM-ANOVA analysis of IBS-QoL the terms found significant to predict the treatment effect were *IBS-subtype*other functional disorders* (Partial Eta Squared (ηp^2) = 0,112 and p = 0,023) and *other functional disorders*treatment* group (ηp^2 = 0,077 and p = 0,019). No potential confounding factors had a significant effect by itself nor changed the conclusions of the model.

In the RM-ANOVA we further explored the difference between fresh vs. frozen vs. placebo in the subgroups with and without additional self-reported functional disorders. Estimated marginal means and confidence intervals for the interaction *treatment group*other functional disorders* are provided in the appendix (Table 2) Important differences in the treatment effect between subgroups were found (Fig. 2A and B). The subgroup without other functional disorder (Fig. 2A), given active treatment (fresh or frozen), shows a profound response from baseline to six months that sustain to twelve months. Same effect is not found in the corresponding placebo group with a small improvement in QoL only from six to twelve months. The participants with other functional disease (Fig. 2B), show a transient treatment effect in both active groups (fresh or frozen) very similar to the placebo group. Finally, we did a post hoc analysis of the individual components of the IBS-QoL using the same variables as in the reduced RM-ANOVA model in a doubly multivariate RM-ANOVA (appendix, Table 2; ηp^2 and p-value for the effect on each sub domain). Treatment group (fresh vs. frozen vs. placebo) combined with other functional disorder (*treatment group*other functional disorders)* had a significant effect on the total score by the subdomains interference with activity, body image, and relationships.

In a post-hoc analysis of FIS we did a repeated measures ANOVA with treatment group (fresh vs. frozen vs. placebo), IBS-subtype and functional comorbidity as predictors. No significant terms were found when the model was reduced. Because fatigue is prevalent in depression, we did a new RM-ANOVA with depression and treatment group as predictors. After removing terms not significant we were left with the term *treatment group (fresh* vs. *frozen* vs. *placebo)*depression* (p = 0,001) as predictor of the treatment effect. None of the potential confounding factors had a significant effect by itself.

In the treatment groups fresh, frozen and placebo there were respectably 5, 4 and 5 participants with self-reported depression at baseline. Because of small sample size in each arm, and the fact that the study was designed to compare active (fresh and frozen combined) to placebo, it was appropriate to combine the fresh and frozen donor FMT group in to one active group and compare it to placebo. Only the term *treatment group (fresh and frozen combined)*depression* had once again a significant effect on the treatment response (ηp^2 = 0,104 and p = 0,005) in the new RM-ANOVA analysis. None of the confounders had a significant effect by itself.

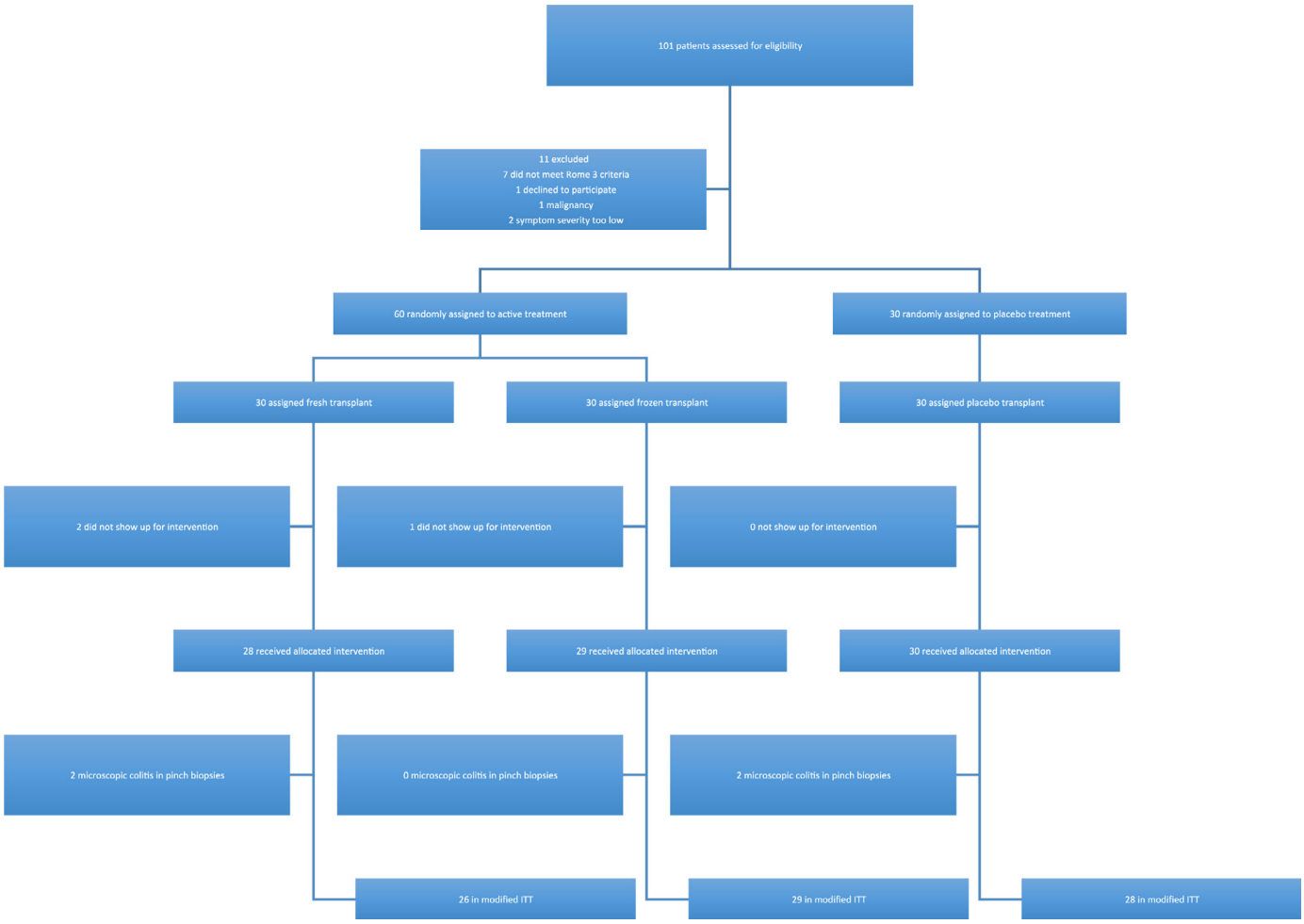

Fig. 1. Study flow chart.

Table 1
Baseline characteristics and demographics.

	Placebo (n = 28)	Active (n = 55)	Fresh (n = 26)	Frozen (n = 29)
Age (years)	45 (34 to 57)	44 (33 to 54)	44 (35 to 54)	43 (26 to 54)
Sex				
Women	19 (68%)	36 (65%)	18 (69%)	18 (62%)
Men	9 (32%)	19 (35%)	8 (31%)	11 (38%)
IBS subtype				
IBS-M	15 (54%)	24 (44%)	12 (46%)	12 (41%)
IBS-D	13 (46%)	31 (56%)	14 (54%)	17 (59%)
Time with IBS (years)	10 (6 to 16)	10 (5 to 19)	15 (4 to 19)	10 (5 to 22)
Depression Ŧ	5 (18%)	9 (16%)	5 (19%)	4 (14%)
Functional comorbidity*	9 (32%)	14 (26%)	7 (27%)	7 (24%)
FIS at inclusion				
Total score	61 (32 to 96)	42 (16 to 78)	42 (26 to 79)	42 (16 to 80)
Score below threshold MCII	6 (21%)	15 (27%)	6 (23%)	9 (31%)
Score in IBS with depression	102 (79 to 129)	109 (56 to 123)	71 (25 to 123)	114 (90 to 144)
Score in IBS without depression	51 (20 to 80)	40 (15 to 60)	40 (22 to 64)	38 (15 to 58)
IBS QoL at inclusion				
Total score	46 (39 to 60)	60 (39 to 74)	61 (33 to 70)	58 (44 to 76)
Score below threshold for MCII	1 (4%)	2 (4%)	1 (4%)	1 (4%)
Score in IBS with functional comorbidity	38 (24 to 46)	56 (35 to 66)	60 (33 to 65)	52 (36 to 78)
Score in IBS without functional comorbidity	56 (44 to 66)	61 (44 to 76)	62 (32 to 79)	60 (49 to 75)
FODMAP before FMT (g/day) Ţ	0,0 (−4 to 4,7)	0,0 (−6,9 to 4,9)	–	–

Data are median (IQR) or n (%), IBS = irritable bowel syndrome, FODMAP = fermentable oligosaccharides, disaccharides, monosaccharaides, and polyols, MCII = minimally clinically important improvement, FIS = fatigue impact scale, IBS-QoL = irritable bowel quality of life.

* Self reported at inclusion; includes fibromyalgia, chronic fatigue syndrome, jaw- and pelvic pain syndromes, ŦSelf reported at inclusion, ŢCalculated from the 5-day dietary record.

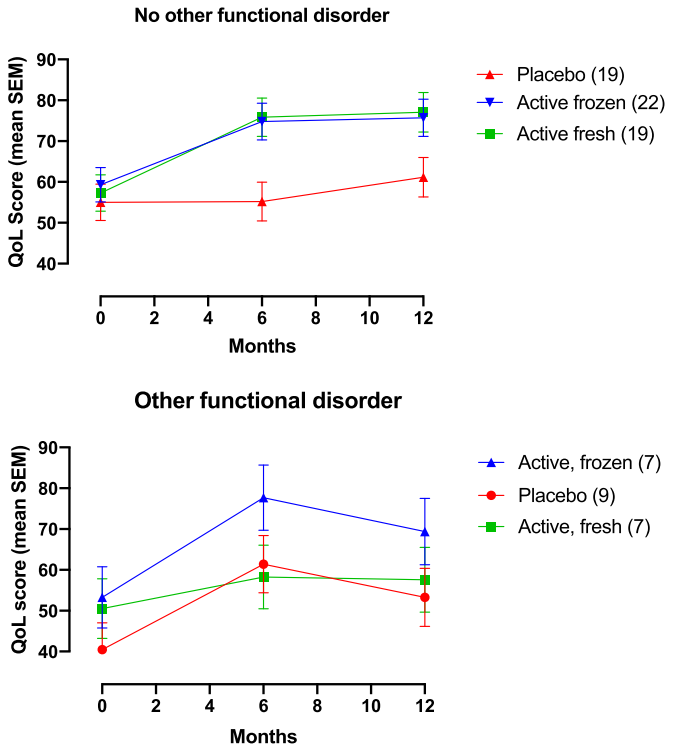

Fig. 2. The repeated time course of the treatment effect in the fresh, frozen and placebo group when functional comorbidity and treatment group are combined, in the term *treatment group*functional comorbidity*, as predictors. The time-course is in estimated marginal means by the IBS-QoL with the standard error in each time point. Fig. 2a is the treatment effect in the subgroup with no other self-reported functional disorders at baseline. Fig. 2b is the treatment effect in the subgroup with functional comorbidity (other than IBS) at baseline. (Number of participants in corresponding treatment group).

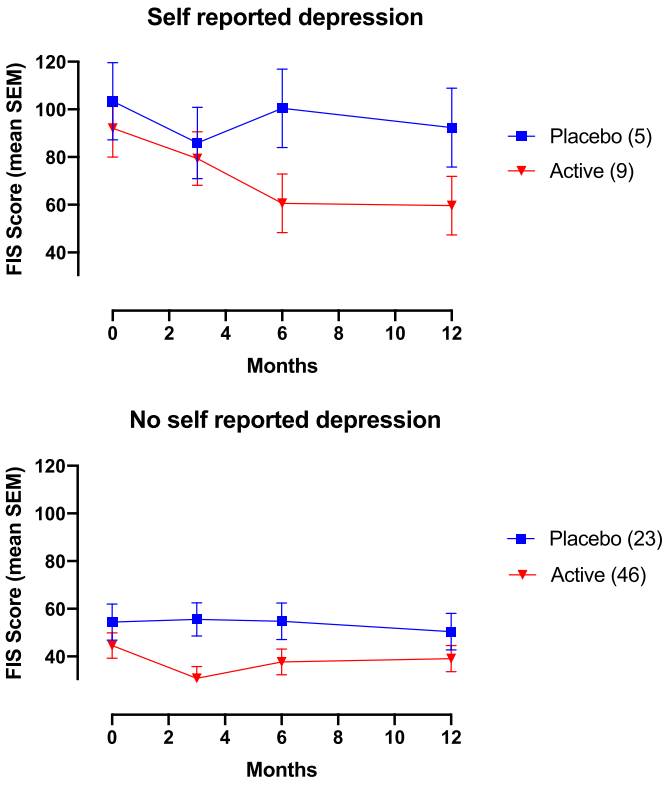

Fig. 3. The repeated time course of the treatment effect active (fresh and frozen combined) and placebo group when depression and treatment group are combined, in the term *treatment group*depression*, as predictors. The time-course is in estimated marginal means by the FIS with the standard error at each time point. Fig. 2a is the treatment effect in the subgroup with self reported depression at baseline. Fig. 2b is the treatment effect in the subgroup without self reported depression at baseline. (Number of participants in corresponding treatment group).

In the RM-ANOVA we further explored the difference in treatment response between the subgroup with and without self-reported depression at baseline and found important distinctions (Fig. 3A and B). Estimated marginal means and confidence intervals for the *interaction treatment group*depression* are provide in the appendix (Table 4) The subgroup with self-reported depression (3A) at baseline shows a treatment response that sustains from baseline and to three, six and twelve months, whereas the placebo response in the same subgroup is low. The subgroup without self-reported depression (3B) shows a treatment effect from baseline to three months that relapses and becomes almost indistinguishable from the effect in the corresponding placebo group.

Finally, we did a breakdown of the individual components of the FIS using the same predictors as in the reduced RM-ANOVA analysis in a doubly multivariate RM-ANOVA (appendix Table 5, ηp^2 and p-value for the effect on each sub domain). Treatment group (active vs. placebo with fresh and frozen donor FMT as one group) combined with depression (*treatment group*depression*) had a significant effect on the total FIS score by all three subdomains (physical, cognitive and social).

4. Discussion

We have presented secondary outcome results from our previously published RCT on FMT in IBS. Results show a clinical effect on QoL and fatigue six months after treatment, with waning effect from six to twelve months. In addition, results mirror our earlier reported findings of the treatment effect on gastrointestinal complaints by the IBS-SSS [5]. Consistency is found in the time course of the treatment response from baseline and until 12 months, and in terms of the predictors that determines the FMT effect. This supports the suggestion that IBS may entail pathophysiologic subgroups extending beyond the current phenotypic subtyping based on stool frequency and consistency [12,13]. Treatment group alone did not have a significant effect by itself on the time-course of the treatment response in QoL, nor in fatigue. This shows that the additional variables (other functional disorders, IBS-subtype and depression) associated with the treatment effect are important. In this context, and for a further discussion, it is very interesting how certain predictors for the FMT effect suggest subgroups with sustainable treatment response that do not only relate to IBS subtype.

Numbers needed to treat (NNT) for an improvement in QoL, six months after treatment, is five and equal to the NNT for relief in gastrointestinal complaints by the IBS-SSS previously reported in these patients [5]. The breakdown of the RM-ANOVA shows that the subdomains most responsive to treatment were *interference with activity, body image and relationships* (appendix, Table 2). Interference with activity includes bothered by how much time spent on the toilet, staying near toilet and worrying about losing control of bowels, getting less done, avoiding stressful situations, strenuous activity and long trips because of bowel problems. Body image includes limitations on what to wear, feeling fat, sluggish and unclean because of bowel problems. Relationships include limitations in interactions with strangers, uncomfortable talking about and feeling that the closes relationships are affected by bowel problems.

In our previously reported results (the effect of FMT on gastro intestinal complaints by the IBS-SSS), the difference in treatment response between fresh and frozen donor FMT (favoring frozen), was clearly due to the confounding effect from participants with additional functional disorders [5]. Same pattern is found in the RM-ANOVA of the treatment response in QoL (Fig. 2A and B). Comorbidity with other functional disorders is associated with a high placebo response and waning treatment effect on QoL (Fig. 2B), whereas IBS without functional comorbidity is associated with a lasting treatment effect and a less pronounced placebo effect (Fig. 2A). As a high and

lasting placebo response often is an issue in clinical trials testing the treatment effect in IBS, this finding should be investigated in future trials [14,15]. Other studies have previously reported an over-representation of somatization disorder in the subgroup of IBS with concomitant somatic comorbidity [16]. A dominating placebo response and lack of a long-term effect in the subgroup with other functional disorders may be attributed to a somatization tendency were FMT have very little effect. Results suggest a lasting FMT effect in IBS without functional comorbidity, whereas IBS with other functional disorders have less benefit. This claim, however, warrants further studies. We hypothesize that additional presence of self-reported functional disorders is a surrogate measure for somatization tendency.

A significant minimal clinically relevant difference in fatigue between active and placebo treatment was only found six months after treatment. However, there were only 73% in the active and 76% in the placebo group with a fatigue score higher than the threshold for minimal clinically relevant difference [2]. Thus making the current dataset less sensitive for changes in FIS score compared to that of QoL and gastrointestinal complaints in this and the previous report, respectively [5]. In addition, the RM-ANOVA suggests that it is mainly the participants with self-reported depression at baseline that experiences a sustaining decrease in fatigue by FMT. A significant difference between active and placebo by the chi square analysis is found six months after treatment when the effect peaked in this subgroup (Fig. 3A). The breakdown of the RM-ANOVA showed responsiveness to treatment in all three subdomains (physical, cognitive and social fatigue) (appendix, Table 3).

Our findings support previous studies suggesting that depression in IBS originates from the gut and not the brain in a subgroup of IBS. In about half of cases IBS symptoms are found to start first and psychological distress developing later [13]. Moreover, a randomized controlled trial from 2017 found an improvement in depression score and altered brain activity in IBS from treatment with a probiotic [17]. The RM-ANOVA of IBS with depression (from the interaction *treatment group*depression*) show a lasting treatment effect from baseline and until twelve months that supports benefit of FMT for fatigue in this subgroup particularly (Fig. 3A). Fatigue, or lack of energy, is one of the hallmarks for depressive disorders [18]. This study also points to a link between fatigue and depression in IBS, as the mean fatigue score at baseline was approximately twice as high in IBS with self-reported depression compared to IBS without (Table 1). It is elusive whether the effect on fatigue is an improvement in depression, or improvement in fatigue as a symptom of IBS. It is important to bear in mind the small sample size of IBS with depression with nine (16,4%) and five (17,9%) participants in the active and placebo group respectively (Table 1). Therefore, our notions of a possible treatment effect on self-reported depression in IBS should be explored in future studies.

The IBS cohort in this study confirms previous findings with fatigue as a common complaint in IBS, and it is the first study that shows improvement of fatigue in IBS from targeting the microbiota by FMT [19]. We hypothesize that fatigue in this study was a surrogate measure for depression as this was the only predictor, in addition to treatment group, that determined the treatment effect. There is support in the literature for the involvement of the microbiota in depression, which could explain why a treatment effect mainly was found in the subgroup with self-reported depression.

We have identified four other randomized controlled trials testing the effect of FMT in IBS [20–23]. Capsulated FMT has not shown any benefits [20,21]. Whereas one study with nasojejunal FMT [23] and one with colonoscopic [22] favored donor FMT compared to placebo (autologous) by a mean improvement in self-reported adequate relief and decrease in gastrointestinal symptom rating-IBS respectively. However, the differences were not significant. The two studies had a lower number of participants than ours, which suggest that the lack of significant findings were caused by underpowered trials.

Route of administration could have an effect on the outcome. The bacterial population increase from the stomach to the colon [24]. In addition, the fermentation of FODMAP's takes place in the colon. This fermentation process is suggested to be involved in IBS pathophysiology [25,26]. Upper delivery of transplants [23], and capsulated FMT [20,21] may lead to an increase of the bacterial population in parts of the digestive system that is not favorable, causing symptom aggravation. In addition, pre-processing from passing through the digestive system may have an impact on how the transplant engraft and influence the colonic fermentation. We, and Holster et al. [22], delivered the transplants to the colon so that the microbiota could engraft in its natural habitat without any pre-processing from the digestive system. The pooled effect of donor FMT by colonoscopic delivery was found significantly in favor of donor FMT in a recent review [27]. In addition, transplants were not prepared by the same technique, did not have the same content (e.g. glycerol concentration), the freeze thaw cycle transplants were exposed to was probably not the same, neither was the amount of feces in transplants, the number of administrations differed, bowel lavage before treatment was not performed in all the studies and the study populations were selected by different criteria. These are important differences that could influence the treatment effect. Finally, only 14 of 664 genera conform to a core microbiota of the gut, so the donor microbiota was probably very different between studies [28]. However, a full comparison on how our results relates to different studies needs a more thorough discussion that is beyond the scope of this manuscript.

The main strength of this study is the thorough characterization of participants at baseline and the long-term follow up that allowed for new findings regarding possible sustainable effects of FMT treatment in subgroups. This study has a number of weaknesses. Of the most important we highlight; first, we do not yet have analysis of the microbiota to support the findings. Second, we used a mix of feces from two donors and we can, therefore, not investigate if there was a donor specific effect. Third, present results are based on secondary endpoints that were not elaborated with a complete statistical analysis plan. However and as planned for, the same statistical analysis as for the reporting of the primary endpoint is applied [5]. This is also consistent with earlier reporting of IBS-related QoL [29]. Forth, change in diet (including probiotics) and/or medications may have influenced the results, however point measures of these parameters did not reveal any change over time as earlier reported [5]. The safety profile is not assessed in this study, but in our previous reported results there were not found any serious adverse events related to the fecal matter in the transplants [5]

5. Conclusion

In conclusion the findings of the FMT effect in QoL and FIS is consistent with the effect on gastro-intestinal complaints earlier reported. The effect on QoL is significant at six months, but not maintained at twelve months. The effect on fatigue is significant at six, but not at three and twelve months. Additional analysis suggests a FMT effect that is maintained until twelve months in subgroups of IBS for both QoL and fatigue. However, the findings must be confirmed in larger studies.

Authors contributions

Funding acquistion: PHJ, PCV, FH, RG; Conceptualization: PHJ, GH, RG PCV; Data curation: PHJ, FH, PCV; Formal anlysis: PHJ, RG; Investigation: PHJ, PCV, FH, RG; Methodology: PHJ, FH, PCV, RG; Project administration: PHJ, RG, FH; Writing orgifinal draft: PHJ, RG, FH, PCV; Writing review and editing: PHJ, PCV, RG, PC.

Declaration of Competing Interest

We declare no competing interests.

Appendix

Tables A1–A5.

Table A1
Baseline IBS-QoL and FIS score, including corresponding subdomain score. Data are mean score (standard deviation). The p-value is the difference between active and placebo score compared in an independent sample T-test.

	Active	Placebo	P-value
IBS-QoL total score	57,7 (19,1)	49,2 (20,6)	0,067
Dysphoria	58,9 (23,8)	47,5 (25,0)	0,046
Interference with activity	49,6 (22,1)	40,1 (24,0)	0,074
Body image	57,4 (25,1)	47,8 (25,0)	0,102
Health worry	63,5 (16,5)	58,3 (16,5)	0,182
Food avoidance	41,1 (26,9)	39,6 (27,8)	0,816
Social reaction	64,5 (22,5)	58,7 (24,6)	0,818
Sexual	60,9 (28,8)	58,5 (30,6)	0,141
Relationships	68,0 (21,8)	58,9 (25,5)	0,093
FIS total score	52,3 (40,3)	63,2 (40,0)	0,249
Cognitive	13,2 (11,1)	15,4 (8,9)	0,380
Physical	13,1 (10,4)	15,5 (10,9)	0,326
Social	26,0 (20,2)	32,3 (21,1)	0,190

Table A2
Estimated marginal means (and confidence interval) from the interaction treatment group (fresh vs. frozen vs. placebo)*other functional disorders.

	Fresh donor FMT	Frozen donor FMT	Placebo	Fresh donor FMT	Frozen donor FMT	Placebo FMT
	With baseline functional disorders			Without baseline functional disorders		
Baseline	51 (36–65)	53 (38–68)	40 (27–54)	57 (48–66)	59 (51–68)	55 (46–64)
6m	58 (43–74)	78 (62–94)	61 (47–75)	76 (66–85)	75 (66–84)	55 (46–65)
12M	58 (42–73)	69 (53–86)	53 (39–68)	77 (67–87)	76 (67–85)	61 (52–71)

Table A3
A doubly multivariate repeated-measures ANOVA of all seven subdomains in IBS-QoL based on the same terms as in the repeated time course of the treatment effect. Partial Eta Squared and (p-value) are corrected by the Greenhouse-Geisser method for all seven subdomains. Patient group (fresh donor vs. frozen donor vs. placebo autologous FMT) combined with other functional disorders (patient group*other functional disorders) have significant effect in the subdomains interference with activity, body image and relationships. *<0,0005.. IBS-QoL = irritable bowel quality of life.

	Dysphoria	Interference with activity	Body image	Health worry	Food avoidance	Social reaction	Sexual	Relationships
Time	0,266 (*)	0,296 (*)	0,270 (*)	0,222 (*)	0,167 (*)	0,211 (*)	0,060 (0,013)	(0,153) (*)
Patient group* Other functional disorders	0,059 (0,318)	0,116 **(0,026)**	0,136 **(0,008)**	0,079 (0,129)	0,081 (0,124)	0,081 (0,126)	0,089 (0,086)	0,137 **(0,006)**
Other functional disorders* IBS-subtype	0,061 (0,060)	0,069 **(0,043)**	0,068 **(0,040)**	0,052 (0,093)	0,022 (0,484)	0,056 (0,078)	0,062 (0,054)	0,098 **(0,005)**

Table A4
Estimated marginal means (and confidence interval) from the interaction treatment group (active vs. placebo)*depression.

	Active and baseline depression	Placebo and baseline depression	Active without depression	Placebo without depression
Baseline	92 (68–119)	103 (71–135)	45 (34–55)	54 (39–69)
3 months	79 (57–101)	86 (56–116)	31 (21–41)	56 (42–70)
6 months	61 (36–85)	100 (68–133)	38 (27–49)	55 (39–70)
12 months	60 (35–84)	92 (59–125)	39 (28–50)	50 (35–66)

Table A5
A doubly multivariate repeated-measures ANOVA of all three subdomains in FIS based on the same terms as in the repeated time laps of the treatment effect. Partial Eta Squared and (P-values) are corrected by the Greenhouse-Geisser method for all seven subdomains. Patient group (fresh and frozen donor FMT combined vs. placebo autologous FMT) combined with other functional disorders (patient group*depression) have significant effect in the subdomains interference with activity, body image and relationships. FIS = fatigue impact scale.

	Physical	Cognitive	Social
Time	0,059 **(0,004)**	0,043 **(0,027)**	0,062 **(0,005)**
Patient group*depression	0,102 **(0,004)**	0,096 **(0,009)**	0,094 **(0,011)**

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Funksjonelle tarmlidelser		
1. Hvor ofte har du hatt ubehag eller smerte i mageregionen de siste 3 månedene?	0. Aldri 1. Mindre enn en dag i måneden 2. En dag i måneden 3. To til tre dager i måneden 4. En dag i uken 5. Mer enn en dag i uken 6. Hver dag	
2. For kvinner: Har du hatt dette ubehaget eller denne smerten kun under menstruasjon og ikke på andre tidspunkt?	0. Nei 1. Ja 2. Gjelder ikke for meg da jeg er forbi overgangsalderen (menopause) eller jeg er mann	
3. Har du hatt dette ubehaget eller disse smertene i 6 måneder eller mer?	0. Nei 1. Ja	
4. Hvor ofte har du hatt hard eller klumpete avføring de siste 3 månedene?	0. Aldri eller sjelden 1. Av og til 2. Ofte 3. Nesten alltid 4. Alltid	Alternativ skala: 0. Aldri eller sjelden 1. Omtrent 25 % av gangene 2. Omtrent 50 % av gangene 3. Omtrent 75 % av gangene 4. Alltid, 100 % av gangene
5. Hvor ofte har du hatt løs, grøtaktig eller vandig avføring de siste 3 månedene?	0. Aldri eller sjelden 1. Av og til 2. Ofte 3. Nesten alltid 4. Alltid	Alternativ skala: 0. Aldri eller sjelden 1. Omtrent 25 % av gangene 2. Omtrent 50 % av gangene 3. Omtrent 75 % av gangene 4. Alltid, 100 % av gangene
6. Hvor ofte har ubehaget eller smerten blitt bedre eller helt borte etter at du har hatt avføring?	0. Aldri eller sjelden 1. Av og til 2. Ofte 3. Nesten alltid 4. Alltid	
7. Hadde du oftere avføring da ubehaget eller smerten begynte?	0. Aldri eller sjelden 1. Av og til 2. Ofte 3. Nesten alltid 4. Alltid	
8. Hadde du sjeldnere avføring da ubehaget eller smerten begynte?	0. Aldri eller sjelden 1. Av og til 2. Ofte 3. Nesten alltid 4. Alltid	
9. Hadde du løsere avføring da ubehaget eller smerten begynte?	0. Aldri eller sjelden 1. Av og til 2. Ofte 3. Nesten alltid 4. Alltid	
10. Hvor ofte hadde du hardere avføring da ubehaget eller smerten begynte?	0. Aldri eller sjelden 1. Av og til 2. Ofte 3. Nesten alltid 4. Alltid	

SPØRRESKJEMA OM IRRITABEL TARM SYNDROM (IBS)

Navn: _____ **Fastlegens navn:** _____

Adresse: _____ **Legesenter:** _____

_____ **Adresse:** _____

_____ _____

Telefon: _____ **Telefon:** _____

Fødselsdato: _____

Sivilstatus: Enslig / Gift / Skilt / Enke / Enkemann / Samboer

Yrke: _____ **Kjønn:** **Mann** ☐ **Kvinne** ☐

Etnisk bakgrunn: Kaukasisk (hvit) / Afrikansk / Asiatisk / Samisk

Fars yrke (selv om han er pensjonist): _____

INSTRUKSJONER

Dette skjema er laget for å registrere hvor alvorlig du er plaget av ditt irritabel tarm syndrom (IBS). Det er vanlig at plagene varierer over tid, så vi ber deg vennligst om å besvare spørsmålene basert på hvor plaget du er for tiden – (det vil si de siste 10 dagene). All informasjon vil bli behandlet strengt fortrolig.

1. På spørsmål hvor ulike svaralternativ er mulige, vennligst sett kryss over det svaret som passer deg best.

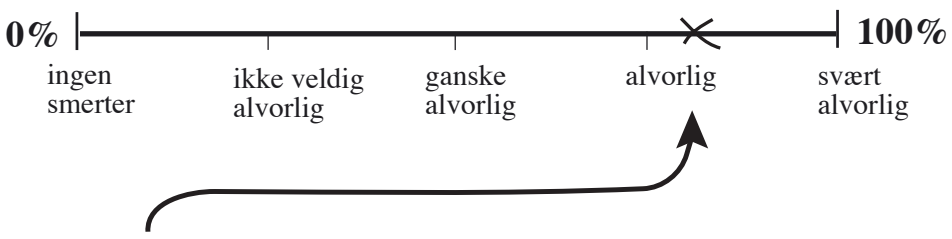
2. Noen spørsmål krever skriftlig svar.

3. Noen spørsmål krever at du setter et kryss på en linje, noe som gir oss muligheten til å bedømme alvorlighetsgraden av et bestemt problem.

For eksempel:

Hvor alvorlig har smertene dine vært?

Vennligst sett et kryss (X) på linjen for å markere hvor alvorlig smerten din har vært



Dette svaret betyr at smertene dine har vært omtrent 80 prosent alvorlig.

DEL 1: GRADERING AV ALVORLIGHET

Fylles ikke ut:

SCORE

1. a) ***Er du for tiden plaget med magesmerter?*** **JA** **NEI**

Sett kryss over JA eller NEI

b) ***Hvis ja, hvor alvorlig er magesmertene dine?***

Vennligst sett et kryss (X) på linjen for å markere hvor alvorlig plagene dine er.

0% —— **100%**

ingen smerte	ikke veldig alvorlig	ganske alvorlig	alvorlig	svært alvorlig

c) ***Vennligst angi antall dager du har smerter i løpet av en 10 dagers periode.***

Eksempel: 4 betyr at du har smerter i magen i 4 av 10 dager.
Hvis du har smerter i magen hver dag, skriver du 10.

Antall dager med smerte _____ x10 = ☐

2. a) ***Er du for tiden plaget med utspilt mage?* (oppblåsthet eller stinnhet)*** **JA** **NEI**

Sett kryss over JA eller NEI

*(*kvinner ser bort fra plager relatert til menstruasjonen)*

b) ***Hvis ja, hvor alvorlig er denne utspiltheten (oppblåstheten/stinnheten)?***

Vennligst sett et kryss (X) på linjen for å markere hvor alvorlig plagene dine er.

0% —— **100%**

ikke utspilt	ikke veldig alvorlig	ganske alvorlig	alvorlig	svært alvorlig

3. ***Hvor fornøyd er du med avføringsvanene dine?***

Vennligst sett et kryss (X) på linjen for å markere hvor fornøyd du er.

0% —— **100%**

svært fornøyd	ganske fornøyd	misfornøyd	svært misfornøyd

4. ***I hvor stor grad føler du at ditt irritable tarm syndrom påvirker eller forstyrrer livet ditt generelt sett?***

Vennligst sett et kryss (X) på linjen for beskrivelsen som passer best.

0% —— **100%**

ikke i det hele tatt	ikke mye	ganske mye	fullstendig

Translation approved by the Rome Foundation

IBS alvorlighetsskår: ☐

DAGENS DATO:_____ _____ _____
DAG MÅNED ÅR

DELTAKERS/PASIENTS ID:

VENNLIGST LES DETTE NØYE

PÅ DE FØLGENDE SIDENE VIL DU FINNE UTSAGN OM TARMPROBLEMER (IRRITABEL TARM-SYNDROM) OG HVORDAN DE PÅVIRKER DEG.

FOR HVERT UTSAGN BER VI DEG VELGE DET SVARET SOM PASSER BEST TIL DEG, OG **SLÅ EN RING RUNDT** TALLET FORAN SVARET DITT.

HVIS DU ER USIKKER PÅ HVA DU SKAL SVARE, BER VI DEG SVARE SÅ GODT DU KAN. **DET FINNES INGEN RETTE ELLER GALE SVAR.**

SVARENE DINE VIL BLI BEHANDLET STRENGT KONFIDENSIELT.

HVIS DU HAR NOEN SPØRSMÅL, BER VI DEG KONTAKTE:

OPPGI NAVN OG ADRESSE PÅ SENTER HER

Hvordan du føler deg

Vi ber deg tenke over livet ditt **den siste måneden (de siste 30 dagene)**, og se på utsagnene nedenfor. Hvert utsagn har fem ulike svar. For hvert utsagn ber vi deg slå en ring rundt tallet foran det svaret som best beskriver dine følelser.

1. Jeg føler meg hjelpeløs på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

   1 IKKE I DET HELE TATT
   2 LITT
   3 MODERAT
   4 EN GOD DEL
   5 SVÆRT

2. Jeg blir flau på grunn av lukt som skyldes tarmproblemene mine. *(Slå ring rundt ett tall.)*

   1 IKKE I DET HELE TATT
   2 LITT
   3 MODERAT
   4 EN GOD DEL
   5 SVÆRT

3. Det er plagsomt for meg at jeg bruker mye tid på toalettet. *(Slå ring rundt ett tall.)*

   1 IKKE I DET HELE TATT
   2 LITT
   3 MODERAT
   4 EN GOD DEL
   5 SVÆRT

4. Jeg føler meg sårbar for andre sykdommer på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

   1 IKKE I DET HELE TATT
   2 LITT
   3 MODERAT
   4 EN GOD DEL
   5 SVÆRT

5. Jeg føler meg tykk/oppblåst på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

   1 IKKE I DET HELE TATT
   2 LITT
   3 MODERAT
   4 EN GOD DEL
   5 SVÆRT

6. Jeg føler at jeg mister kontrollen over livet mitt på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

Vennligst fortsett på neste side

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

7. Jeg føler at jeg gleder meg mindre over livet på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

8. Jeg føler meg utilpass når jeg snakker om tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

9. Jeg føler det er deprimerende å ha tarmproblemer. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

10. Jeg føler meg isolert fra andre på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

Vennligst fortsett på neste side

11. Jeg må passe på hvor mye mat jeg spiser på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

12. Tarmproblemene gjør seksuell aktivitet vanskelig for meg. *(Slå ring rundt ett tall.)*
(Hvis dette er uaktuelt for deg, slå ring rundt "IKKE I DET HELE TATT".)

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

13. Jeg føler meg sint og ergerlig for at jeg har tarmproblemer. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

14. Jeg føler at jeg irriterer andre på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

15. Jeg bekymrer meg for at tarmproblemene mine skal bli verre. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

Vennligst fortsett på neste side

16. Jeg føler meg irritabel på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

17. Jeg bekymrer meg for at folk tror at jeg overdriver tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

18. Jeg føler at jeg får gjort mindre på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

19. Jeg må unngå stressende situasjoner på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

20. Tarmproblemene mine gjør at jeg får mindre lyst på sex. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

Vennligst fortsett på neste side

21. Tarmproblemene mine begrenser hva jeg kan ha på meg. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

22. Jeg må unngå fysisk anstrengende aktiviteter på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

23. Jeg må passe på hva slags mat jeg spiser på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

24. På grunn av tarmproblemene mine er det vanskelig for meg å omgås folk som jeg ikke kjenner godt. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

25. Jeg føler meg slapp og sløv på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

26. Jeg føler at jeg er ”uren” på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

Vennligst fortsett på neste side

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

27. Lange reiser er vanskelige for meg på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

28. Jeg føler meg oppgitt og frustrert over at jeg ikke kan spise når jeg vil på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

29. Det er viktig å være i nærheten av et toalett på grunn av tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

30. Livet mitt dreier seg om tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

Vennligst fortsett på neste side

31. Jeg bekymrer meg for å miste kontrollen over endetarmen og avføringen. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

32. Jeg er redd for at jeg ikke vil klare å få avføring. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

33. Tarmproblemene mine virker forstyrrende på forholdet til mine nærmeste. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT

34. Jeg føler at ingen forstår tarmproblemene mine. *(Slå ring rundt ett tall.)*

1 IKKE I DET HELE TATT
2 LITT
3 MODERAT
4 EN GOD DEL
5 SVÆRT MYE

Spørreskjema om virkninger av tretthet/utmattelse

Pasientnummer: _____ Dato: _____

Nedenfor er det en liste med utsagn som beskriver hvordan tretthet/utmattelse kan føre til problemer i folks liv. Vennligst les hvert utsagn nøye. Slå en ring rundt det tallet som best viser hvor stort problem tretthet/utmattelse har vært for deg **i de siste fire (4) ukene**, inkludert i dag. Vennligst slå en ring rundt ett tall for hvert utsagn og hopp ikke over noen utsagn.

Ring rundt ett tall på hver linje	Ikke noe problem	Lite problem	Moderat problem	Stort problem	Meget stort problem
1. *På grunn av tretthet/utmattelse,* **føler jeg meg mindre oppmerksom og årvåken.**	0	1	2	3	4
2. *På grunn av tretthet/utmattelse*, **føler jeg meg mer isolert fra sosialt samvær.**	0	1	2	3	4
3. *På grunn av tretthet/utmattelse,* **må jeg minske min arbeidsbelastning eller andre forpliktelser.**	0	1	2	3	4
4. *På grunn av tretthet/utmattelse,* **er jeg mer humørsyk.**	0	1	2	3	4
5. *På grunn av tretthet/utmattelse,* **har jeg vansker med å feste oppmerksomheten på noe i lengre perioder.**	0	1	2	3	4
6. *På grunn av tretthet/utmattelse,* **føler jeg at jeg ikke kan tenke klart.**	0	1	2	3	4
7. *På grunn av tretthet/utmattelse,* **arbeider jeg mindre effektivt. (Dette gjelder arbeid både i og utenfor hjemmet).**	0	1	2	3	4
8. *På grunn av tretthet/utmattelse,* **er jeg mer avhengig av at andre hjelper meg eller gjør ting for meg.**	0	1	2	3	4
9. *På grunn av tretthet/utmattelse,* **har jeg vansker med å planlegge aktiviteter på forhånd fordi min tretthet/utmattelse kan komme i veien for dem.**	0	1	2	3	4
10. *På grunn av tretthet/utmattelse,* **er jeg mer klønete og klossete i bevegelsene.**	0	1	2	3	4
11. *På grunn av tretthet/utmattelse,* **merker jeg at jeg er mer glemsk.**	0	1	2	3	4
12. *På grunn av tretthet/utmattelse,* **er jeg mer irritabel og blir lettere sint.**	0	1	2	3	4
13. *På grunn av tretthet/utmattelse,* **må jeg være nøye med hvor ofte og hvor lenge jeg driver med fysiske aktiviteter.**	0	1	2	3	4
14. *På grunn av tretthet/utmattelse,* **er jeg mindre innstilt på å gjøre ting som krever fysisk anstrengelse.**	0	1	2	3	4

Final version of August 2004 – MAPI Research Institute – ID 2302.

Ring rundt ett tall på hver linje	Ikke noe problem	Lite problem	Moderat problem	Stort problem	Meget stort problem
15. *På grunn av tretthet/utmattelse,* **er jeg mindre innstilt på å delta i sosialt samvær.**	0	1	2	3	4

16. *På grunn av tretthet/utmattelse,* **er jeg mindre i stand til å komme meg ut på større eller mindre turer eller reiser utenfor hjemmet.**	0	1	2	3	4
17. *På grunn av tretthet/utmattelse,* **har jeg vansker med å holde det gående med fysiske anstrengelser over lengre perioder.**	0	1	2	3	4
18. *På grunn av tretthet/utmattelse,* **finner jeg det vanskelig å ta avgjørelser.**	0	1	2	3	4
19. *På grunn av tretthet/utmattelse,* **har jeg lite sosialt samvær utenfor mitt eget hjem.**	0	1	2	3	4
20. *På grunn av tretthet/utmattelse,* **er vanlige dagligdagse hendelser stressende for meg.**	0	1	2	3	4
21. *På grunn av tretthet/utmattelse,* **er jeg mindre innstilt på å gjøre noe som krever tankevirksomhet.**	0	1	2	3	4
22. *På grunn av tretthet/utmattelse,* **unngår jeg situasjoner som er stressende for meg.**	0	1	2	3	4
23. *På grunn av tretthet/utmattelse,* **føles musklene mine mye svakere enn de burde.**	0	1	2	3	4
24. *På grunn av tretthet/utmattelse,* **øker mitt fysiske ubehag.**	0	1	2	3	4
25. *På grunn av tretthet/utmattelse,* **har jeg vansker med å ordne opp i eller forholde meg til nye ting.**	0	1	2	3	4
26. *På grunn av tretthet/utmattelse,* **er jeg mindre i stand til å fullføre oppgaver som krever tankevirksomhet.**	0	1	2	3	4
27. *På grunn av tretthet/utmattelse,* **føler jeg meg ute av stand til å oppfylle de krav som folk stiller til meg.**	0	1	2	3	4
28. *På grunn av tretthet/utmattelse,* **føler jeg meg mindre i stand til å forsørge meg selv og familien økonomisk.**	0	1	2	3	4
29. *På grunn av tretthet/utmattelse,* **er jeg mindre seksuelt aktiv.**	0	1	2	3	4
30. *På grunn av tretthet/utmattelse,* **finner jeg det vanskelig å holde orden på tankene mine når jeg driver med ting hjemme eller på jobben.**	0	1	2	3	4
31. *På grunn av tretthet/utmattelse,* **er jeg mindre i stand til å fullføre oppgaver som krever fysisk anstrengelse.**	0	1	2	3	4

32. *På grunn av tretthet/utmattelse,* **er jeg bekymret for hva andre mennesker synes om utseendet mitt.**	0	1	2	3	4

33. *På grunn av tretthet/utmattelse,* **er jeg mindre i stand til å ordne opp i eller forholde meg til ting som har med følelser å gjøre.**	0	1	2	3	4
34. *På grunn av tretthet/utmattelse,* **føler jeg at tankene mine går langsommere.**	0	1	2	3	4
35. *På grunn av tretthet/utmattelse,* **finner jeg det vanskelig å konsentrere seg.**	0	1	2	3	4
36. *På grunn av tretthet/utmattelse,* **har jeg vansker med å delta fullt ut i aktiviteter sammen med familien.**	0	1	2	3	4
37. *På grunn av tretthet/utmattelse,* **må jeg begrense mine fysiske aktiviteter.**	0	1	2	3	4
38. *På grunn av tretthet/utmattelse,* **trenger jeg hyppigere eller lengre hvileperioder.**	0	1	2	3	4
39. *På grunn av tretthet/utmattelse,* **er jeg ikke i stand til å gi så mye følelsesmessig støtte til familien som jeg burde.**	0	1	2	3	4
40. *På grunn av tretthet/utmattelse*, **virker det som om små vansker egentlig er store vansker.**	0	1	2	3	4

5 dagers kostregistrering for REFIT studien

Ved spørsmål ta kontakt med : XXXXX på telefon XXXX

Slik gjør du:

For at vi skal kunne beregne et nøyaktig næringsstoffinntak, er det nødvendig at du noterer så nøyaktig som mulig absolutt ***alt*** du spiser og drikker i løpet av **en xx dagers sammenhengende periode.**

Det er viktig at du spiser slik som du pleier i registreringsperioden.

- Angi klokkeslett for hver gang du spiser eller drikker noe.
- Beskriv mat og drikke så nøyaktig som mulig
    - Brød: type, navn, grovhet, tykkelse på skiver, antall skiver. Evt. rundstykke, knekkebrød…
    - Fett på brødet: type, navn, mengde, lett eller vanlig
    - Pålegg: type, mengde, produktnavn, lett eller vanlig
    - Middag: type kjøtt, fisk kjøttfarse-/fiskeprodukt. Produktnavn. Fettprosent.
    - Frukt og grønnsaker: rå, kokt eller hermetisk
- Beskriv hvordan maten er tilberedt
    - Kokt, bakt, stekt, grillet eller varmet i mikrobølgeovn
    - Hvilken type fett bruker du til matlaging, og mengde? Olje, smør, flytende margarin?
    - Er maten renset for skinn og eller fett? Eks. kylling med skinn? Kotelett med feittrand?
- Hjemmelagede matretter beskrives i detalj, gjerne ved å skrive ned oppskriften bak på arket
- Noter alt tilbehør, som saus, rømme, dressing eller krem, med navn/produsent. Oppgi også om du bruker sukker på gryn, grøt eller i te.
- Få med alle mellommåltider, samt tilfeldig spising og drikke utenom de faste måltidene.
- Kosttilskudd, som tran, vitamintabletter, probiotika og lignende skal også noteres, med navn, produsent og mengde.
- Mengder kan beskrives på følgende måte:
    - Aller helst skal du veie maten og føre mengden opp i gram
    - Hvis du ikke kan veie, kan du angi mengder i husholdningsmål, som teskje, spiseskje, glass, desiliter eller antall, alt ettersom hva som er hensiktsmessig
    - Oppgi størrelse på glass og kopper du bruker i dl

Kl	**Onsdag 14/01/10**	**Produktnavn/produsent**	**Vekt/mengde**
08.30	1 butikkskåret skive kneip	Bakers	30g
	m/skrapet lag margarin	Soft soya	
	3 høvelskiver hvitost, 16% fett	Norvegia, Tine	
	1 stor grapefrukt		200g
	1 stort glass lettmelk	Tine	2 dl
12.00	1 beger fruktyoghurt	Yoplait Dobbel 0%, mango	125g
	1 melkesjokolade	Freia melkerull	77 g
	1 kopp svart kaffe		150g
16.00	Kokt torsk		140g
	3 små poteter, kokt		150g
	3 toppede ss revet gulrot		
	1 ss remulade	Idun	
	2 store glass saft	Lerum uten tilsatt sukker	2 x 2 dl

Kl	.....dag .../.../...	Produktnavn/Produsent	Mengde

Første konsultasjon Sjøkanten:

1) Medikamentliste, inkl reseptbelagt og ikke reseptbelagte anti- diaremedisiner og evt. p-piller

2) Har du brukt antibiotika siste 3 mnd? I så fall hvilke?

3) Tidligere sykdommer, dvs alle sykdommene pasienten er kjent må å ha eller har hatt:

4) Alvorlige allergier, eller allergi mot medikamenter?

5) Har vært plaget med IBS i _____ år / måneder

6) Ledsagende sykdommer (tidligere sykdommer ikke relevant)
   a) Fibromyalgi
   b) Kronisk tretthetssyndom
   c) Kroniske smerter i kjeveleddet
   d) Kroniske bekkenbunnsmerter (Chronic pelvic pain)
   e) Angst
   f) Depresjon
   g) Andre sykdommer som du ser i sammenheng med din IBS_____

7) Hva utløste dine plager
   a) Akutt magesyke (gastroenteritt)
   b) Depresjon/Angst
   c) Antibitokakur
   d) Livskrise
   e) Vet ikke
   f) Andre årsaker, spesifiser_____

8) Coloskopert før

   Antall ganger _____

9) Har du siste måneden forsøkt å behandle plagene dine med livsstilsendringer?
   a) Fysisk aktivitet
   b) kostomlegging
   c) Andre_____

d) Hvis ja, hvilke(t) alternativ har fungert? _____

10) Er det noen matvarer du ikke tåler, i så fall spesifiser?

11) Får du hjelp av noen disse fagruppene?
    a) Psykolog b)Psykiater c) Ernæringsfysiolog d) IBS skole e)allmennlege

    e) Gastroenterolog Alternativ behandler, dersom ja spesifiser:_____

    Spesifiser om det er noen av disse faggruppene du synes er nyttig:

    _____

    Spesifiser om noen av disse faggruppene gjør deg mindre plaget med din IBS:

    _____

12) Behandler du dine plager med medisiner eller kosttilskudd
    Sett en strek under de alternativene som du mener gir bedring. Angi forbruk i gram(g)/miligram (mg)/milliliter (ml)

    a) Antidepresiva_____

    b) Medisiner som motvirker diare _____

    c) Probiotika og kosttilskudd med innhold av bakterier _____

    d) Omega 3 tilskudd _____

13) Har du fått tilbud om noen form for behandling for din IBS?

14) Vitalia
    Puls:_____ BT:_____

    Høyde:_____ Vekt:_____

    BMI:_____

Forespørsel om deltakelse i forskningsprosjektet

Behandling av irritabel tarm gjennom fekal mikrobiotisk transplantasjon ''FMT"

Bakgrunn og hensikt

Dette er et spørsmål til deg om å delta i en forskningsstudie for å undersøke om overføring av avføring fra en frisk giver kan behandle og kurere symptomene og plagene hos mennesker med irritabel tarmsyndrom. Du blir forespurt om å delta i studien siden du oppfyller kriteriene til irritabel tarmsyndrom, IBS.

Vår hypotese er at irritabel tarmsyndrom hos mange mennesker er forårsaket av en ubalanse i den naturlige mikrobielle tarmfloraen. Nyere forskning og flere nye fagartikler støtter vår hypotese. Vi håper å kunne gjenopprette en normal tarmflora nettopp gjennom overføring av fersk avføring fra en frisk person til pasienten.

Studien gjennomføres som et felles forskningsprosjekt mellom allmennlege og spesialist ved sykehuset i Harstad.

Hva innebærer studien?

Alle deltakerne som oppfyller kriteriene på irritabel tarmsyndrom og sier seg villig å delta i studien blir på forhånd undersøkt/screenet, på andre sykdommer i tykktarmen som f.eks betennelse i tarm, kronisk infeksjon i tykktarm og tykktarmkreft.

På legekontoret fyller du ut et spørreskjema i forhold til grad og omfang av dine mageplager. Deretter blir du henvist til magetarm poliklinikken i Harstad. Her blir deltakerne delt opp i en behandlings- og en kontroll gruppe.

Studien gjennomføres som en dobbel blind studie. Det betyr at verken du eller undersøkeren på poliklinikken vet hvilken behandling som blir gitt.

Behandlingsgruppen vil få overført fersk avføring fra en frisk giver. Giveren (donor) blir på samme måte som deltakerne i studien screenet på mulige sykdommer, for å sikre at ikke f.eks uheldige bakterier overføres.

Kontrollgruppen vil få overført placebovæske som ikke inneholder avføring. Det er svært viktig å gjennomføre denne studien med en kontrollgruppe (placebogruppe). På den måten kan vi bekrefte eller avkrefte om behandlingen hjelper.

Overføring av fersk avføring er blitt gjort mange ganger i forbindelse med andre sykdommer i tykktarm uten at det noen gang har ført til alvorlige bivirkninger.

Avføringen eller placebovæsken blir plassert i tykktarmen gjennom et koloskop, som ved en vanlig tykktarmundersøkelse. Dermed får man samtidig inspisert hele tykktarmen.
Alle deltakerne i studien blir tett fulgt opp over en periode av et år.
I oppfølgingsperioden skal det besvares med jevne mellomrom et spørreskjema om grad og omfang av plagene i forhold til irritabel tarmsyndrom.
Skulle behandlingen vise seg å være til nytte, vil selvfølgelig alle deltakerne i kontrollgruppen også få tilbud om slik behandling.
Ønsker du ikke å være med i studien vil du få oppfølging som vanlig for irritabel tarmsyndrom, via din fastlege.

Fekal transplantasjon ved Irritabel tarm syndrom
Harstad 2013

Mulige fordeler og ulemper

Overøfring av fersk frisk avføring er blitt gjort mange ganger og har hittil ikke hatt noen som helst alvorlige bivirkninger.
Vanlige bivirkninger som avføringstrang, oppblåsthet og forbigående magesmerter kan forekomme. Disse symptomene vil gå over i løpet av noen timer. Vi vet ikke om overføring av fersk avføring hjelper ved irritabel tarmsyndrom, men ved andre, mer alvorlige tarmsykdommer har denne behandlingen ofte hatt svært god effekt.
Tykktarmsundersøkelse med koloskop er i dag en vanlig prosedyre som gjøres flere ganger per dag for eksempel i Harstad. På den måten kan tarmen inspiseres og for eksempel betennelsestilstander eller svulst oppdages tidlig. Koloskopi innebærer alltids en liten risiko for å kunne skade tarmslimhinnen eller stikke hull i tykktarm, men dette er svært sjeldent.

Hva skjer med prøvene og informasjonen om deg?

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste.
Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Etter endt oppfølgingstid på et år skal dataene om deg fjernes.
Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte prosjektleder Peter Johnsen på tlf.:90723298 eller Frank Hilpüsch på 95792833.

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som hentes i forbindelse med helsesjekken lagres. I tillegg vil blod-, avførings- og tarmslimhinneprøvene også bli lagret. Etter endt studie – som er anslått til å vare i til sammen 3 år vil alle opplysninger knyttet til deg bli slettet.

Medisinsk avdeling ved Univseritetssykehuset Nord-Norge ved administrerende direktør er databehandlingsansvarlig.

Biobank

Avføringsprøvene som blir tatt vil bli lagret i en forskningsbiobank ved Forskningsgruppe for Gastroenterologi og Ernæring ved Universitetssykehuset Nord-Norge. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken.

Professor Jon Florholmen er ansvarshavende for forskningsbiobanken. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK).

Utlevering av materiale og opplysninger til andre

Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger utleveres til Universitetet i Tromsø og Barents Biocenter i Tromsø.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede opplysninger og destruert innsamlede prøver, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi

Studiet finansieres gjennom Helse-Nord forskningsmidler og vil ikke være avhengig av midler fra personer eller organisasjoner som har økonomiske særinteresser.

Forsikring
Studiedeltakeren er forsikret gjennom Universitetssykehuset i Nord Norge

Informasjon om utfallet av studien

Alle deltakere har rett til å få informasjon om utfallet av studiet og vil få publikasjoner tilsendt per post.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg samtykker til og kunne bli kontaktet dersom det skulle bli aktuelt å gjøre andre analyser eller starte flere prosjekt i tilknytting denne studien

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(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Forespørsel om deltakelse i forskningsprosjektet

Behandling av irritabel tarmsyndrom gjennom fekal mikrobiotisk transplantasjon (FMT)"

Bakgrunn og hensikt

Dette er et spørsmål til deg om å delta i en forskningsstudie for å undersøke om overføring av avføring fra en frisk giver kan behandle og kurere symptomene og plagene hos mennesker med irritabel tarmsyndrom. Du blir forespurt om å delta i studien siden du oppfyller kriteriene til å være donor av avføring. Vår hypotese er at irritabel tarmsyndrom hos mange mennesker er forårsaket av en ubalanse i den naturlige mikrobielle tarmfloraen. Nyere forskning og flere nye fagartikler støtter vår hypotese. Vi håper å kunne gjenopprette en normal tarmflora nettopp gjennom overføring av fersk avføring fra en frisk person til pasienten. Studien gjennomføres som et felles forskningsprosjekt mellom allmennlege og spesialist ved sykehuset i Harstad.

Hva innebærer studien?

Alle deltakerne som oppfyller kriteriene som donor og sier seg villig å delta i studien blir på forhånd undersøkt/screenet på andre sykdommer i tykktarmen som f.eks betennelse i tarm, kronisk infeksjon i tykktarm og tykktarmkreft. I tillegg vil vi kartlegge bakterifloraen i avføringsprøven din for å se om den lar seg overføre til en IBS pasient.

Undersøkelsen skjer ved medisinsk polklinikk UNN Harstad. Dette innebærer en generell helsesjekk og blod og avføringsprøver for å avdekke tilstander som kan virke inn på resultatet. I tillegg vil det bli gjort en sigmoideoskopi der man går inn med en slange i den ytterste del av tykktarmen for å ta en prøve av tarmslimhinnen. Prøven fra slimhinnen sammenlignes med prøvene fra pasientene med IBS for å kartlegge rollen til det lokale immunforsvaret i tarmen ved IBS. Etter undersøkelse ved medisinsk poliklinikk vil du få nærmere tilbakemelding per telefon eller post om du fortsatt er egnet som donor i studiet. Dersom man på noen av undersøkelsene eller prøveresultatene finner avvik som tyder på ikke erkjent underliggende sykdom vil du bli informert om dette per telefon eller brev og oppfordret til å kontakte din fastlege for videre oppfølging.

Som donor må du møte opp på gitte ettermiddager ved Harstad sykehus for å avlevere avføring som skal brukes til å behandle deltakerne i studiet. Du kan bli bedt om å møte opp alt fra 1 til 5 ganger.

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Honorar

Som kompensasjon for tidsbruk i forbindelse med oppmøte ved UNN Harstad får du 500 kr for hver gang du avleverer avføring.

Mulige fordeler og ulemper

Vi vet ikke om overføring av fersk avføring hjelper ved irritabel tarmsyndrom, men ved andre, mer alvorlige tarmsykdommer har denne behandlingen ofte hatt svært god effekt. Tykktarmsundersøkelse med sigmoideoskop er i dag en vanlig prosedyre som gjøres flere ganger per dag for eksempel i Harstad. På den måten kan tarmen inspiseres og for eksempel betennelsestilstander eller svulst oppdages tidlig. Sigmoideoskopi innebærer alltids en liten risiko for å kunne skade tarmslimhinnen eller stikke hull i tykktarm, men dette er svært sjeldent.

Hva skjer med prøvene og informasjonen om deg?
Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Når studien er endt skal navnelisten destrueres og data vil være avidentifisert. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte prosjektmedarbeider Peter Johnsen på tlf.:90723298 eller Frank Hilpüsch på 95792833.

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel A – Personvern, biobank, økonomi og forsikring.

Samtykkeerklæring følger etter kapittel A.

Kapittel A - Personvern, biobank, økonomi og forsikring

Personvern
Opplysninger som hentes i forbindelse med helsesjekken lagres med studienummer som kan identifiseres med en navneliste. I tillegg vil blod-, avførings- og tarmslimhinneprøvene også bli lagret med samme studienummer. Etter endt studie – som er anslått til å vare i til sammen 3 år vil navnelisten bli destruert slik at det ikke vil være mulig å identifisere noen av studiedeltakerne i databasen.

Medisinsk avdeling ved Univseritetssykehuset Nord-Norge ved administrerende direktør er databehandlingsansvarlig.

Avføringsprøvene som blir tatt vil bli lagret i en forskningsbiobank ved Forskningsgruppe for Gastroenterologi og Ernæring ved Universitetssykehuset Nord-Norge. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken.

Professor Jon Florholmen er ansvarshavende for forskningsbiobanken. Det biologiske materialet kan bare brukes etter godkjenning fra Regional komité for medisinsk og helsefaglig forskningsetikk (REK).

Utlevering av materiale og opplysninger til andre
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Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede

opplysninger og destruert innsamlede prøver, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi
Studiet finansieres gjennom Helse-Nord forskningsmidler og vil ikke være avhengig av midler fra personer eller organisasjoner som har økonomiske særinteresser.

Forsikring
Studiedeltakeren er forsikret gjennom Universitetssykehuset i Nord Norge.

Informasjon om utfallet av studien
Alle deltakere har rett til å få informasjon om utfallet av studiet og vil få publikasjoner tilsendt per post.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)__

09.01.2014 REK nord

Forskningsprosjekt
Behandling av irritabel tarmsykdom ved fekal mikrobiotisk transplantasjon
Vurdering:

Vi viser til deres tilbakemelding av 02.12.13 på komiteens merknader 3.10.13

Rek har vurder at tilbakemeldingen for det vesentlig er i tråd med de merknader komiteen gav i sitt utsettelsesvedtak den 3.10.13.

REK viser bare presisere at vi legger til grunn at Valle er kontaktperson for forskningsansvarlig som er UNN, og ikke den forskningsansvarlige slik det står i samarbeidsavtalen.

I de vedlagte forespørsler ser vi at honorering nå er lagt inn som et eget punkt, men i formuleringen "tapt arbeidsfortjeneste", må tas vekk fordi en honorering skal være en kompensasjon for utgifter til evt. reise, tidsbruk ect.

Etter fullmakt er det fattet slikt:

Vedtak:

Med hjemmel i helseforskningsloven § 10 og forskningsetikkloven § 4 godkjennes prosjektet.