Diploma thesis

Fecal Microbiota Transplantation

Overview of Indications, Efficacy and Safety

submitted by

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Graz, October 30th, 2025

Statutory Declaration

I hereby solemnly declare that I have written the present thesis independently and without any external assistance, that I have used no sources other than those indicated, and that all passages taken either verbatim or in substance from the sources used are clearly marked as such.
Furthermore, I declare that, if Artificial Intelligence (AI) tools were employed in the generation and/or correction of specific text, such use was conducted in accordance with ethical principles, academic integrity, and the regulations of my university. The use was subsequently disclosed and appropriately documented in a transparent manner.

Graz, October 30th, 2025 Johannes Zach m.p.

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Finally, I would like to thank all study participants whose involvement made this research possible.

Abstract

Background

Fecal microbiota transplantation (FMT) has emerged as a promising therapeutic approach for a range of medical indications and has begun to be integrated into clinical guidelines for rCDI. Its application varies with different protocols concerning stool preparation, the way of transplantation and donor selection criteria.
Encouraging evidence has been reported regarding the efficacy of FMT across various gastrointestinal and metabolic disorders. Limitations arise due to the lack of standardized protocols which hinders the comparability of different studies.
While short-term safety data are reassuring, there is a need for systematic, long-term surveillance of adverse events (AEs) and serious adverse events (SAEs). Moreover, the heterogeneity of patient populations in existing studies complicates the interpretation of results, underscoring the necessity for more uniform patient selection criteria.

Objective and Methods

The objective of this thesis was to provide an overview of FMT and to analyze clinical data of 118 patients who received FMT at the Medical University of Graz. The primary focus of the analysis was the identification and evaluation of potential adverse events and serious adverse events.
The patient cohort included individuals with various medical indications. Due to the absence of standardized international protocols, institution-specific protocols were developed and retrospectively reviewed. Additionally, body mass index data was collected.
A second focus was placed on the review of five publications in which FMT was employed for the treatment of various disease conditions. The primary emphasis of the review was the occurrence and classification of AEs and SAEs following FMT.

Results

A follow-up period of at least 37 days was conducted for all patients at the Medical University of Graz, during which no AEs or SAEs were reported. Most post-

procedural symptoms were mild and most likely attributable to the colonoscopy itself.

Across the selected studies mentioned above, reported AEs were also mostly mild and self-limiting. SAEs were rare and, in most cases, not directly attributable to the FMT itself. Nevertheless, most reviewed publications stressed the limitations of their data, particularly with respect to the reliability of safety assessments.

Conclusion

Summing up it can be said that the findings suggest that FMT is a safe therapeutic option with low rates of AEs and rare SAEs. However, future research must focus on large scale clinical trials with harmonized protocols.

Zusammenfassung

Hintergrund

Fäkale Mikrobiotatransplantation (FMT) zeigt sich als wirksamer Ansatz zur Behandlung vieler medizinischer Krankheitsbilder und ist für rCDI bereits in den klinischen Leitlinien verankert. Es gibt keine einheitlichen Protokolle in Bezug auf die Art der Transplantation und die Kriterien der Spender*innen-Auswahl.
Was die Wirksamkeit von FMT betrifft, zeigen sich vielversprechende Ergebnisse bezüglich diverser gastrointestinaler Erkrankungen und Stoffwechselprobleme.
Da es zurzeit keine standardisierten Protokolle für die Durchführung einer FMT gibt, ist die Möglichkeit eines Vergleichs verschiedener Studien eingeschränkt.
Während die kurzfristigen Sicherheitsdaten vielversprechend sind, gibt es einen dringenden Bedarf nach systematischen langfristigen Studien der unerwünschten Ereignisse (AEs) sowie der schwerwiegenden unerwünschten Ereignisse (SAEs).
Die Heterogenität der Studienteilnehmer*innen erschwert eine eindeutige Interpretation der Ergebnisse. Dies unterstreicht die Notwendigkeit, einheitliche Kriterien für die Auswahl der Patient*innen zu erstellen.

Ziele und Methoden

Ziel der vorliegenden Arbeit war es, einen Überblick über FMT zu bieten und die klinischen Daten von 118 Patient*innen, welche an der Medizinischen Universität Graz mit FMT behandelt wurden, zu analysieren. Der Schwerpunkt lag auf der Identifizierung und Evaluierung möglicher AEs und SAEs.
Die Kohorte umfasste Patient*innen mit heterogenen Indikationen. Da es keine standardisierten internationalen Protokolle gibt, wurden eigene Protokolle für die Medizinische Universität Graz erstellt und rückblickend ausgewertet.
Zusätzlich wurden fünf Publikationen rezensiert, in denen FMT zur Behandlung unterschiedlicher Krankheiten beschrieben wird. Der Fokus der Beurteilung bezog sich auf das Auftreten und die Klassifizierung von AEs und SAEs.

Ergebnisse

Innerhalb der Patient*innen-Kohorte der Medizinischen Universität Graz konnten in einem Beobachtungszeitraum von mindestens 37 Tagen keine nennenswerten

AEs und SAEs konstatiert werden. Die meisten Symptome waren mild ausgeprägt und vermutlich auf die Koloskopie selbst zurückzuführen.
Auch innerhalb der rezensierten Studien wurde nur über leichte Nebenwirkungen berichtet. SAEs waren selten und standen meist in keinem direkten Zusammenhang mit der FMT-Behandlung. Allerdings wurde in allen Publikationen betont, dass die vorhandenen Daten bezüglich einer zuverlässigen Aussage über AEs und SAEs stark eingeschränkt waren.

Schlussfolgerung

Zusammenfassend kann gesagt werden, dass FMT eine sichere therapeutische Methode mit nur wenigen AEs und seltenen SAEs zu sein scheint. Zukünftige Studien müssen sich jedoch auf klinische Untersuchungen mit einheitlichen Protokollen fokussieren.

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List of Abbreviations

AE	adverse event
BMI	Body Mass Index
CD	Chron´s disease
CIBD	chronic inflammatory bowel disease
E. coli	Escherichia coli
FGD	functional gastrointestinal disorder
FMT	Fecal Microbiota Transplantation
GIT	gastrointestinal tract
GM	gut microbiome
GVHD	graft-versus-host disease
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
ÖGGH	Austrian Society of Gastroenterology and Hepatology
PPI	proton pump inhibitor
rCDI	recurrent Clostridioides difficile infection
SAE	serious adverse event
UC	ulcerative colitis

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The human gastrointestinal tract and its microbiome

The human gastrointestinal tract (GIT) is colonized by countless arts of bacterial species. There are more than 1014 kinds of bacteria, fungi and viruses referring to the tract. About 100 trillion bacterial cells are part of the human intestinal tract. This gut microbiome (GM) weighs about 1.5 kg and has more than 3.3 million genes. Its functions are numerous. Digestion, assisting in nutritional provision and protecting the body from pathogens are just a few of them. This shows that the GM plays an essential role for the well-being of the human body and its organs. The GM is formed from birth and differs between individuals, but it is very stable and resilient over time. However, there are plenty of factors that can interfere with this microbiome such as environmental factors. These are for example diet, probiotics, prebiotics, pathogens like viruses or bacteria, drugs, especially antibiotics.
Several diverse groups of diseases are closely related to the GM. These include infectious conditions (like Clostridioides difficile infection or infectious gastroenteritis), autoimmune diseases (such as allergic diseases, diabetes, inflammatory bowel disease), some general diseases (overweight, functional gastrointestinal disorders), and behavioral diseases.(1–5)
As mentioned above the GM varies from individual to individual, however, there are still some identifiable fundamental compositions which emerge into a “healthy” microbiome. Specific basic patterns among commensal bacteria are identifiable in such a “healthy” microbiome. Bacteria that colonize the human gut can be described using different levels of granularity with taxonomy. Taxonomy uses units like phylum, class, order, family, genus, species, or strain to define the kingdom of bacteria. One common way of describing a GM is to analyze the bacterial microbiome in the gut carried out with the help of the phylum level. At this level the GM is mainly characterized by four types. These are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. In a “healthy” GM these four types comprise up to 99% of intestinal microbiota.
Firmicutes, which are gram positive bacteria, include more than 200 genera (the most essential ones are Ruminococcus, Clostridium and Lactobacillus). They make up between 60% to 80% of the GM.
Bacteroides are gram negative bacteria like Prevotella accounting for 20-30% of

the microbiota.

Thirdly there are Actinobacteria which constitute around 10% of the GM. This group primarily includes the genus Bifidobacterium.

Finally, there are Proteus bacteria as Escherichia coli (E. coli) or Enterobacteriaceae.(2,3)

Displayed below is a taxonomic tree that systematically organizes the primary bacterial groups comprising the human gut microbiome. The hierarchical model provides a visual representation of the complex organization within the microbiome, tracing from the broad classification of bacteria down to specific genera within each phylum.

This taxonomic tree serves as a structured overview, offering insights into the diversity and functional specialization of bacteria which contributes to the dynamic ecosystem within the human gastrointestinal tract. Understanding these relationships is fundamental for advancing research into microbiome-related health and disease outcomes.

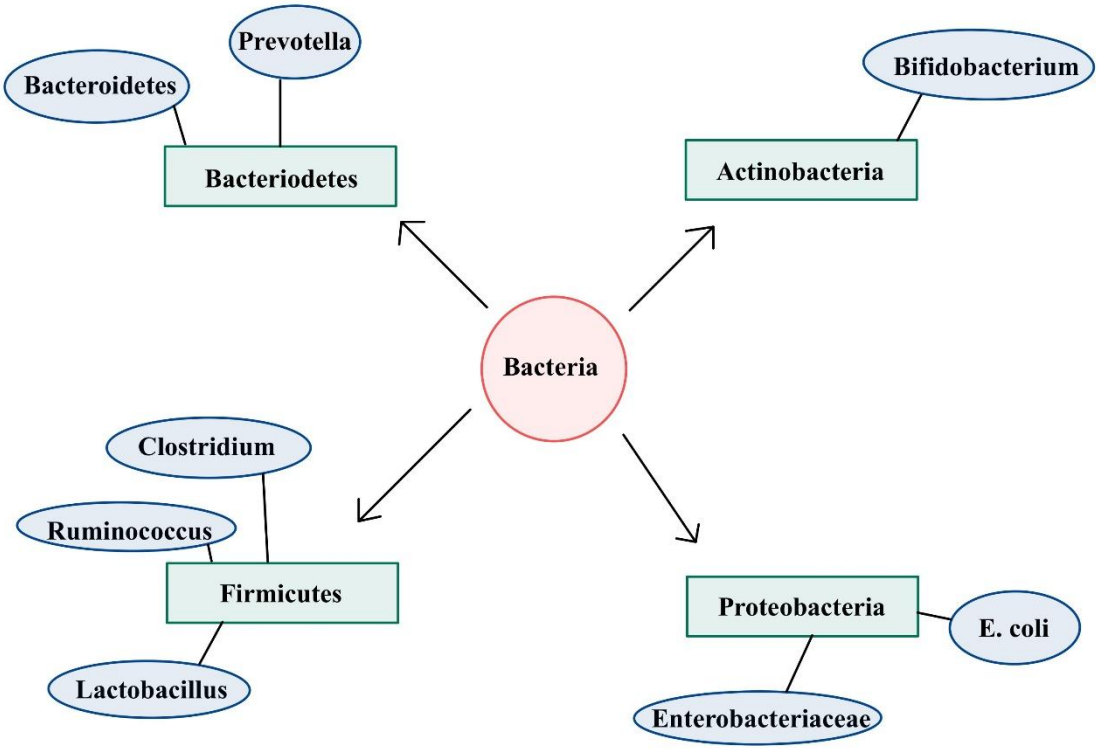

Figure 1: Taxonomic tree

Dysbiosis in the human gut microbiome

Dysbiosis of the human GM is defined as a state of the GM that differs from a "healthy" or normal state (eubiosis). Changes in the composition of the microbiome can include reduction in species diversity or alterations in the ratio of "healthy" to "unhealthy" microbes.(6–8)

Dysbiosis is a broad term as the microbiome of the gut can be considered unhealthy in various individualistic ways. A common example of dysbiosis is an increased relative amount of Proteobacteria such as E. coli and Klebsiella species. There is no gold standard method for identifying dysbiosis, however, several indices have derived from studies conducted among patients with recurrent Clostridioides difficile infection (rCDI).

One way to diagnose dysbiosis lies in dividing the total number of Proteobacteria by the total number of overall bacterial strains.(3)

An alternative approach is called the Microbiome Health Index. This method analyzes the dispersion within the different phyla levels which refer mainly to Firmicutes, Bacteroidetes, and Proteobacteria. This approach compares the abundances of bacteria belonging to the Clostridia and Bacteroidia classes (Firmicute and Bacteriodete phyla) which associate with healthy functions to the abundances of Bacilli and Gammaproteobacteria (Firmicute and Proteobacteria phyla) which associate with pathogenesis.(3)

As mentioned above such changes in the GM can occur due to different factors or because of various influences and may lead to a wide range of different diseases. Lack of colonization resistance may lead to overgrowth of pathogenic bacteria such as Clostridium difficile as a result of the loss of commensal bacteria. CDI is a well-known and frequently described consequence of dysbiosis which can happen after antibiotic exposure.(3)

Moreover, there could be a functional immune compromise because of the fact that a "healthy" GM plays a crucial role in the immune modulation in a state of homeostasis. A higher amount of Proteobacteria may lead to an increased risk of gut inflammation, which in turn can enlarge the risk of systemic infection such as bacteremia.

In addition to its well-known role as a cause of CDI, dysbiosis is also associated with numerous other states and diseases of several organ systems in the human

body. Among them are immune-mediated/autoimmune diseases like inflammatory bowel disease (Crohn´s disease and ulcerative colitis), metabolic/cardiovascular disorders as obesity, hypertension or type 2 diabetes mellitus, some kinds of cancer like colorectal ones or liver cancer, neuropsychiatric conditions such as Parkinson´s disease, Alzheimer´s disease and depression, infectious diseases like CDI or human immunodeficiency virus and others like chronic kidney disease, liver disease or even sepsis.(3,5,9)

Restoring dysbiosis

Nowadays there are some therapeutical ways which intent to restore an "unhealthy" GM into its "healthy" state. There are several methods with which dysbiosis of the GM can be treated.
The first one tries to eliminate the inciting factor. This one is very common and can be achieved by the use of antibiotics.
The second method is administered by so called pre- or probiotics.

Prebiotics

Prebiotics are used as a source of nutrition for gut bacteria. Most of them consist of carbohydrate groups and are mainly oligosaccharide carbohydrates. They play a significant role in human health. Upon ingestion, prebiotics are selectively fermented by commensal gut microbiota, leading to the generation of short-chain fatty acids such as acetate, butyrate and propionate. These metabolites contribute significantly to intestinal health, immune modulation, metabolic homeostasis and may even influence neuropsychological functioning.(10–13)
Prebiotics are naturally present in a variety of dietary food sources, including asparagus, onions, honey, bananas, tomatoes, soybeans, milk, beans and many others. However, due to their low natural abundance in foods, prebiotics are commonly produced on an industrial scale to meet nutritional and functional demands.(3,14,15)

Probiotics

Probiotics are not nutrition but live microorganisms which can be taken in and intent to improve the GM and thus its host. They exert their health-promoting effects via a variety of mechanisms, including the competitive exclusion of pathogenic bacteria, the synthesis of beneficial metabolites, the enhancement of the intestinal mucosal barrier and the modulation of immune responses – particularly through the induction of regulatory T cells and the upregulation of anti-inflammatory cytokines.(16–18)

Commonly, probiotics include Lactobacillus, Bifidobacterium, or Saccharomyces species.(19) These kinds of treatments may be beneficial in some cases. However, they do not take into account the immense diversity of the GM and are not recommended by guidelines for the primary or secondary infection prevention of CDI.(3)

Moreover, most probiotic strains have been developed for their resistance to low gastric pH, resulting in numerous variants with unknown physiological properties. The wide diversity of microbial combinations complicates comparisons and creates the misleading impression of a uniform drug class, leading to inappropriate prescriptions.

Additionally, the lack of independent studies hinders understanding of the specific physiological effects associated with individual strains.(20)

Fecal microbiota transplantation

The third method is called Fecal Microbiota Transplantation (FMT). In this case it is tried to change the GM of the recipient in order to normalize its composition and to achieve a therapeutic benefit via the stool of a healthy donor.(21)
FMTs can be traced back to the fourth century. In China human stool was used in patients with severe diarrhea at that time. Later, in the sixteenth century, fresh or fermented fecal suspensions were applied to patients with diarrhea, constipation, and abdominal pain. In 1958 FMT was used to treat patients with pseudomembranous colitis. FMTs were then put into action in the veterinary medicine setting. Acquapendente was an Italian surgeon who created the term “transfaunation” which means transference of gastrointestinal content from a healthy to a sick animal. In 1989 the first record of FMT used in a non-infectious disease was published. The procedure was performed on a 45-year-old with refractory ulcerative colitis. The patient experienced complete and enduring clinical recovery following treatment. As the clinical application of FMT transitioned from infectious to non-communicable disorders, the range of FMT applications expanded rapidly. Additionally, emerging insights linking the GM to extraintestinal diseases would further expand the clinical utilization of FMTs. In 2013 the first randomized controlled trial was performed. This study revealed that patients with recurrent CDI treated with FMT via duodenal infusion of donor feces had a better outcome than patients only treated with antibiotics. Since this event the number of studies showing high cure rates of recurrent CDI cases after being treated with FMTs has increased rapidly. (1,3,21,22)

Donor Selection

In order to ease the occurrence of adverse events, it is recommended to implement stringent donor screening tests for FMTs. Both the guidelines from the United States and the European Consensus Conference advocate the use of a donor questionnaire as to assess exclusion and inclusion criteria. A compiled list exemplifying such inclusion and exclusion criteria, which is used at the Medical University of Graz, can be found further below.

In addition to the questionnaire it is advised that donors undergo a follow-up interview on the day of donation, so that any donor behaviors immediately preceding the stool donation can be identified.

Furthermore, standardized screening protocols for donors should be developed to minimize the risk of infection transmission from donor to recipient. An appropriate donor should undergo comprehensive blood and stool examinations within four weeks prior to donation.

At the Medical University of Graz, a comprehensive microbiological stool analysis is conducted to detect viruses, including noroviruses, rotaviruses, and adenoviruses, as well as bacteria, such as Salmonella, Campylobacter, Yersinia, Shigella, and Clostridioides difficile, and various parasites.

Due to shared environmental factors, a spouse is frequently selected as a donor, as it is hypothesized that this choice reduces the risk of infection transmission. Additionally, a close relative is often selected as a donor. This practice is based on the hypothesis that the similarity of microbial species between relatives will result in exhibiting greater tolerance of the adaptive immune system within the mucosal immune system towards the donor´s GM.

Despite these hypotheses, there is a lack of clinical evidence establishing a correlation between the donor and the outcomes of FMT treatments. Non-related FMT donations may sometimes confer advantages, particularly in instances where genetics potentially contribute to the diseases, such as in inflammatory bowel disease.

The interval between screening and donation is another pivotal factor. The FMT French Group recommends minimizing the timeframe to a maximum of 21 days, so that the risk of contamination is mitigated.

Hence, meticulous, and comprehensive donor screening is imperative. Regrettably this endeavor is also associated with substantial efforts and costs.(1,23,24)

Inclusion Criteria
Age between 18 and 65
Informed consent
Exclusion Criteria
Bacterial infectious disease
Allergic disease
Metabolic disease
Autoimmune disease
Neurodegenerative disease
Mental illness
Neoplastic disease
Premature cardiovascular disease (men before age 55: women before age 65)
Some kinds of medications
Gastrointestinal infection in the last 3 months
Antibiotic therapy in the last 6 months

Table 1: Example of inclusion and exclusion criteria of donors

Recipient preparation

Prior to undergoing FMT, patients require thorough briefing. They should abstain from any form of antibiotic intake or administration 12 - 48 hours preceding the procedure.(1,25) However, recipients must receive antibiotic therapy for one to two weeks before the first FMT. This is because a reduction in the body´s own microbiome leads to a better growth of the donor microbiome. Depending on the study protocol, different antibiotics are chosen for this purpose, such as the gut-selective antibiotic rifaximin.(26–28)

Furthermore, patients need to be adequately prepared for FMT, a process akin to other endoscopic procedures. It is essential that the colon is devoid of visible fecal material contaminations prior to the donor feces infusion to ensure a robust engraftment.(29) Some studies also advocate the usage of loperamide one hour prior to FMT to facilitate retention of transplanted stool within the intestines for a minimum of four hours.(30–32)

Similar to the donor, the recipient should undergo a comprehensive questionnaire assessing inclusion and exclusion criteria. These criteria are tailored to the specific condition being treated with FMTs. The table below shows an example of such criteria for participants in a pilot study for FMT in patients diagnosed with irritable bowel syndrome at the Medical University of Graz.

Inclusion Criteria
Age between 18 and 65
Informed consent
Diagnosis of irritable bowel syndrome (according to the"S3 Guideline on Irritable Bowel Syndrome" by the AWMF)
Negative tissue transglutaminase
Unremarkable colonoscopy/gastroscopy
Unremarkable histology of the colonoscopy
Calprotectin under 300

Exclusion criteria
Pregnancy
Breastfeeding
Hemophilia/bleeding disorder
Anticoagulant medication (except simple platelet aggregation inhibitors)
Severe chronic illness
Participation in another clinical study
Secondary motility disorder
Major abdominal surgery

Table 2: Example of inclusion and exclusion criteria of the recipient (irritable bowel disease)

Fecal preparation

The optimal preparation method for stool intended for FMTs remains to be fully elucidated. Various strategies involve the use of freshly collected stool or frozen samples.(1) Presently, several trials and meta-analyses indicate comparable efficacy between both approaches in the management of recurrent or refractory CDI.(33–35)

Fresh specimens should be processed within six hours of donor collection. Approximately 50 grams of fecal material are homogenized with about 150 ml of sterile normal saline solution. Subsequently, the mixture undergoes further filtration to remove large particulate matter, thereby minimizing the risk of endoscope channel obstruction.(1)

Depending on the study protocol, the transplant is administered immediately after preparation or stored in freezers at -80 degrees Celsius for later use. Before storage in freezers, glycerol can be added to stabilize the bacterial cell membranes.

On the day of the FMT treatment, the frozen stool samples are thawed in a water bath at 37 degrees Celsius. After that, normal saline solution may be added to the mixture depending on local experience and study protocol. It is imperative to refrain from subjecting the material to multiple freeze-thaw cycles, and the infusion must be started within a maximum of six hours post-thawing.(1)
Nevertheless, delineating the precise volume of fecal infusion proves to be a formidable task. Greater quantities have exhibited superior outcomes in patients with CDI, whereas amounts below 50 grams have led to a four-fold higher risk of therapeutic failure.(1)

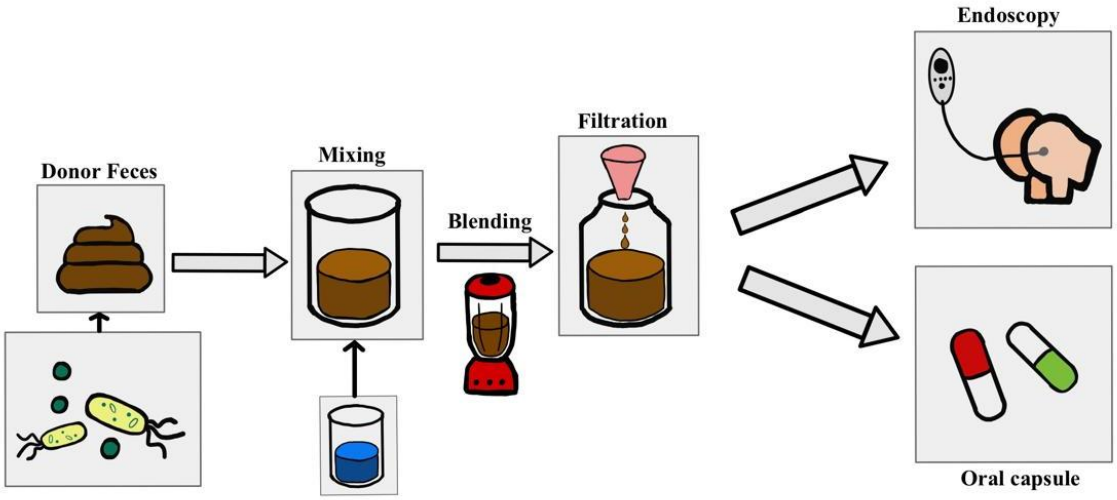

Figure 2: Diagram of Fecal Microbiota Transplantation process

Methods of stool delivery

The fecal transplantation can either be delivered via the upper or lower GI tract. The upper GI tract includes the application via esophagogastroduodenoscopy or via nasogastric, nasojejunal or nasoduodenal tube. Furthermore, the fecal transplant can be encapsulated and ingested as oral capsules. However, the most common method is transmission through colonoscopy via the lower GI tract. Besides that, retention enema can be used to administer the FMT in the distal colon.(36)
The selection of method depends on multiple factors. Broadly speaking, patients affected by inflamed intestinal conditions can be administered via the upper

gastrointestinal route. (1) However, it is imperative to be aware of the fact that unpleasant sensations may ensue during the placement of the tube. Furthermore, there exists a risk of aspiration, and additionally this method precludes the assessment of colonic mucosa and the collection of mucosal samples.

FMTs administered through the lower gastrointestinal tract exhibit enhanced efficacy in recolonizing the entire colon with desired bacterial species. Apart from that, colon lavage is anticipated to diminish the presence of residual organisms and spores. Nevertheless, this approach is comparatively costly, fraught with risks, and entails a high degree of invasiveness.(1)

FMTs via retention enema represents a cost-effective and less invasive alternative to colonoscopy. Nonetheless, it is significant to note that with this approach, the delivery of donor stool is confined to the distal colon, excluding distribution throughout the entire colon.(36)

The use of oral capsules for FMTs offers the assets of minimal invasiveness and high patient acceptability. However, limitations include the associated expenses, the need for a local stool bank in order to be able to produce a high amount of capsules and the significant burden of ingesting multiple capsules each day.(1)

Numerous studies have been conducted to compare the various methods, yielding diverse findings.(37–39) While certain investigations indicate parity between upper and lower gastrointestinal tract deliveries, others demonstrate superior clinical resolution rates associated with lower gastrointestinal tract interventions over upper gastrointestinal tract approaches in patients afflicted with CDI.

However, investigations suggest that FMTs administered via oral capsules exhibit similar efficacy in preventing recurrent CDI when compared with colonoscopic delivery.(37)

Nevertheless, it is essential to point out the fact that the selection of the appropriate delivery variant must be predicated upon a multitude of factors. These factors encompass the patients´ physiological state, their individual response to therapeutic intervention, and the efficacy thereof. Furthermore, site specific expertise guides the decision.(1)

Indications of Fecal Microbiota Transplantation

At the moment, FMT is established in present clinical guidelines only for treating rCDI.(40) However, there are various other potential indications which still have to be examined. These indications exhibit considerable diversity concerning the nature of the ailment, encompassing immune-mediated/autoimmune disorders, infectious diseases, metabolic syndromes, and various neurological conditions. (1,22)

Despite the numerous possible uses of FMTs, the current focus in clinical practice lies on treating recurrent or refractory CDI cases.(22) Due to remission rates above 90%, several expert guidelines have established FMTs as the primary therapeutic modality for individuals afflicted with recurrent and refractory CDI.(41) Moreover, FMT has been accepted as the sole indication approved by the United States Food and Drug Administration since 2013.

In addition to CDI treatments, FMTs are of significant interest for patients with IBDs, as well. Several trials have demonstrated promising outcomes.(42) A clinical study showed that in patients with ulcerative colitis, after having undergone FMT treatment, the intestinal mucosa appeared to experience repair, leading to improved outcomes in both ulcerated and healthy mucosa. Additionally, there was a notable reduction in the number of acute and chronic inflammatory cells.(43) However, these outcomes have not been as favorable as those observed in CDI treatment. Therefore, modified strategies for FMT application, including intensive-dosing multidonor FMTs have been performed. These strategies have demonstrated favorable treatment effects in IBD patients.

Furthermore, it has turned out that FMTs are a more cost-effective alternative compared to antibiotics.(44) Notably, the treatment of ulcerative colitis shows considerable promise.

However, its efficacy in Crohn´s disease remains limited at this stage.

Another approach concerning the utilization of FMTs lies in the treatment of functional bowel disorders. Several meta-analyses and cohort studies have demonstrated improvements in bowel movement among patients with irritable bowel syndrome as a consequence of FMT treatment.(45–47) Nonetheless, studies have also reported findings suggesting that FMT may not provide a consistent or significant improvement in global symptoms of IBS.(48) It has been

observed that delivery into the small intestine results in a long-term higher response rate and facilitates sustained colonization of beneficial bacteria compared to the delivery into the large intestine.

Moreover, repeated administration of FMTs has yielded superior effects on symptoms and quality of life compared to a single FMT.(49)

In addition to gastrointestinal diseases, recent years have seen growing interest in the application of FMTs as far as the treatment of extra-intestinal conditions is concerned. Several studies have highlighted the potential of modifying the gut microbiome as a therapeutic approach for obesity and metabolic syndrome.(50–52) In the therapeutic intervention making use of FMTs with patients afflicted with metabolic syndrome, a crucial augmentation in insulin sensitivity among these patients has been conclusively ascertained.

Studies have identified dysbiosis in the microbiome of patients with type 2 diabetes mellitus.(53–56) It is known that the GM is involved in the regulation of bile acid metabolism and its metabolites. Notable metabolites include short-chain fatty acids, lipopolysaccharides, and trimethylamine oxide, all of which play crucial roles in human metabolic functions. Therefore, targeting the GM by an intervention of FMTs presents a potential therapeutic avenue for the treatment of type 2 diabetes mellitus.(57)

A dysbiosis in the GM results in the production of different metabolites and cytokines. These molecules induce inflammation, influence the blood-brain-barrier and brain volume, and may function as pseudo-neurotransmitters. Consequently, these factors disrupt brain physiology and neuronal function, potentially leading to a range of neurological disorders, including those characterized by demyelination or neuropsychiatric manifestations.(58)

Thus, it is conceivable that therapeutic effects of FMT may extent to conditions such as multiple sclerosis, autism spectrum disorders, infections caused by multidrug-resistant pathogens and even multi-organ dysfunction in critically ill patients.(59–62) However, these hypotheses are primarily supported by preliminary evidence from case reports and animal studies and should therefore be interpreted with caution until validated by robust clinical trials.

Moreover, insights obtained by several animal models and clinical trials have suggested a favorable impact on melanoma when immunotherapy is administered in connection with FMTs. In this approach, stool from patients with malignant

melanoma who have responded to PD-L1 therapy is transplanted to patients who did not respond to immunotherapy. PD-L1, a protein predominantly expressed on the surface of tumor cells, plays a critical role in immune regulation, primarily by suppressing immune responses.(63,64) As such, it is a key target in cancer therapy.(65) Following transplantation, recipients exhibit enhanced response to PD-L1 therapy like their donors.(1)

A list of conditions undergoing evaluation for FMTs as a therapeutic principle is shown in the table below.

Gastrointestinal Diseases	Metabolic Diseases	Neuropsychiatric Diseases	Neoplastic Diseases	Hematological and inflammatory disorders	Infectious diseases
Clostridiodes difficile infection	obesity	Parkinson's disease	Tumor cachexia	Graft vs host disease	Colonization with multidrug-resistant bacteria
Ulcerative colitis	diabetes mellitus	Alzheimer's disease	Renal cell carcinoma	Checkpoint inhibitor-induced colitis	Coronavirus disease 2019 (COVID-19)
Crohn´s disease	Primary hypertension	Depression/bipolar disorders	Loss of response to checkpoint inhibitor therapies	Psoriatic arthritis	Human immunodeficiency virus (HIV)
Pouchitis		Autism	Malignant melanoma	Rheumatoid arthritis	Recurrent urinary tract infections
Microscopic colitis		Multiple sclerosis	Prostate cancer	Ankylosing spondylitis	Myalgic encephalomyelitis/ chronic fatigue syndrome
Radiation enteritis		Guillain-Barré syndrome	Acute myeloid leukemia	Alopecia areata	
Helicobacter pylori infection		Amyotrophic lateral sclerosis	Pancreatic carcinoma	IgA nephropathy	
Small intestinal bacterial overgrowth (SIBO)		Epilepsy	Metastatic colorectal carcinoma	Peanut allergy	
Irritable bowel syndrome/chronic constipation		Insomnia	Hepatocellular carcinoma	Atopic dermatitis	
Checkpoint inhibitor-induced colitis			Bronchial carcinoma	Sjögren's syndrome	
Acute-on-chronic liver failure			Mesothelioma	Immune thrombocytopenia	
Hepatic encephalopathy			Glioblastoma	Chronic rhinosinusitis	
Steatohepatitis					
Primary sclerosing cholangitis					
Chronic hepatitis B infection					
Acute pancreatitis					

Table 3: Conditions undergoing evaluation for FMTs as a therapeutic principle (22)

Contraindications of Fecal Microbiota Transplantation

As of present, there are no absolute contraindications of FMT.(66) Nonetheless, meticulous consideration of individual patient parameters and pertinent clinical contexts is of extraordinary significance in determining the appropriateness of FMT intervention. A comprehensive risk-benefit analysis is essential and may necessitate rigorous risk assessment procedures.(1)
Thus, so far FMT has only been established for treating rCDI in several clinical guidelines.(40)

Mechanism of FMT in recurrent CDI

The precise mechanism by which FMT aids in recurrent CDI remains unclear. However, several hypotheses have been proposed. (67)
Firstly, there is the reconstitution of microbial alpha diversity which enhances colonization resistance against Clostridioides difficile. Experiments have demonstrated a relative increase in the frequency of Bacteroidetes and a reduction in the frequency of Proteobacteria after having undergone FMT treatment. This has been accompanied by a composition and diversity profile more akin to that of the donors. Subsequently, functional alternation ensued, facilitating colonization resistance.(68)
Secondly, it is assumed that there is direct competition of Clostridioides difficile with commensal microbiota introduced via FMT. The diverse array of GM engages in competitive interactions for ecological niches and nutrient resources, potentially culminating in the production of bacteriocins. These bacteriocins exhibit either bacteriostatic properties, impeding the growth of rival bacteria, or bactericidal effects, leading to their elimination. This competitive condition, coupled with bacteriocin production, can modulate the population dynamics of Clostridioides difficile, thereby impeding its colonization and proliferation within the GIT.(69)
Thirdly, alternations in bile acid metabolism have to be mentioned. It has been reported that bile acid metabolism plays a role in the pathogenesis of recurrent CDI. Bile acids, derived from cholesterol and synthesized in the liver, undergo modification in the colon by indigenous gut microbiota, resulting in the production

of secondary bile acids. Bile acids exert regulatory effects on the composition of the GM and can modulate the life cycle of Clostridioides difficile.
Taurocholate, primary bile salt, is commonly employed in Clostridioides difficile growth media, while lithocholic acid, a secondary bile acid, acts as an inhibitor of Clostridioides difficile spore germination. Studies have observed elevated concentrations of primary bile acids and bile salts in pre-FMT fecal samples of patients suffering from rCDI, with secondary bile acids being nearly absent. Conversely, post-FMT stool samples exhibit concentrations of secondary bile acids akin to those found in non-CDI donor samples.
This suggests a perturbation in bile acid metabolism in recurrent CDI patients and underscores the potential of FMTs to rectify recurrent CDI due to normalizing both microbiome composition and bile acid metabolism.(70)
Fourthly, it is believed that stimulation of the mucosal immune system is able to restore gut barrier function. The intestinal barrier exhibits dynamic responses to diverse stimuli and serves as a pivotal defense mechanism against pathogenic invasion. Integral to this defense is the immune system. Experimental investigations utilizing murine models have elucidated that perturbations in the intestinal barrier can engender heightened bacterial colonization of the intestinal surface and subsequently activate the immune system. The immunological response to Clostridioides difficile infections manifests itself variably depending on strain-specific attributes and host-specific factors. Severe presentations of Clostridioides difficile infection correlate with intestinal inflammation typified by distinct biomarkers in fecal matter. FMTs are capable of modulating the intestinal barrier by delivering pivotal signals which conduct epithelial cell regeneration within the intestine and facilitate the synthesis of mucus and antimicrobial peptides.(69)

Adverse events in FMTs

The primary aim of this study focuses on the examination of adverse events associated with FMT. While FMT has demonstrated considerable success and generated significant optimism, concerns regarding the safety of the procedure persist.

Research indicates that a significant number of patients remain uncertain about considering FMT as a viable therapeutic option. A major source of concern for many patients is the potential risk associated with insufficient screening of donors for pathogenic microorganisms, which may lead to adverse consequences.(71) Investigating these safety issues presents challenges due to the lack of standardized protocols for FMT administration.

Variability exists in several aspects, including the question whether the stool is stored freshly or frozen, the choice of donors, who may be either family members or unrelated individuals, and the absence of uniform screening protocols for both donors and recipients.

Moreover, challenges exist in the follow-up process. Many follow-up protocols lack completeness. Additionally, only a limited number of patients has been monitored over an extended period of time following FMT. Consequently, it is difficult to draw scientifically validated conclusions about potential long-term effects.(71)

The absence of standardized protocols in the administration of FMTs shows substantial implications for clinical practice. This lack of uniformity contributes to significant variability in treatment outcomes, raises serious safety concerns, and introduces considerable challenges in clinical decision-making. Additionally, it hinders the process of research by impeding the ability to conduct large-scale, controlled studies and to compare findings resulting from different studies. The associated inconsistency does not only affect the reliability of clinical results but also complicates efforts to establish evidence-based guidelines and to improve overall patient care.

Definition of adverse events

In order to adequately describe an adverse event, it is essential to first establish a clear definition of the term. In the vast majority of clinical studies, side effects are systematically categorized in two distinct classifications: adverse events (AE) and severe adverse events (SAE).(72) This systematic classification serves a crucial role in the research context, as it facilitates a more nuanced and comprehensive understanding of the potential risks associated with the intervention or procedure being examined.

By distinguishing between these two categories, researchers and clinicians can better assess the safety profile of the treatment and its implications for patient care.

The definitions of these two classifications are as follows:

Adverse events (AEs): *"AE is defined as any untoward medical occurrence in a patient after administration of FMT that does not necessarily have a causal relationship with this treatment. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with FMT, whether or not related to the FMT."(72)*

Serious adverse events (SAEs): *"A SAE is any adverse experience occurring during or after FMT that results in any of the following outcomes: death, life-threatening experience, inpatient hospitalization or prolongation of existing hospitalization, persistent or significant disability or incapacity, congenital anomaly birth defect, or an important medial event."(72)*

Research for studies with adverse events in FMT

In order to carry out this study, a comprehensive and systematic literature research was conducted using a variety of search strategies in PubMed to identify the most relevant and recent publications. The first search strategy involved the use of the following search terms: (Faecal microbiota transplantation) AND (safety) AND (inflammatory bowel disease), with the aim of finding studies related to safety and adverse events of FMTs as far as inflammatory bowel disease is concerned. As a broad and inclusive approach is demanded, additional research was carried out using the following combinations of terms: (Faecal microbiota transplantation) AND (safety) AND (recurrent Clostridiodes), (Faecal microbiota transplantation) AND (safety) AND (graft-versus-host disease) and (Faecal microbiota transplantation) AND (safety) AND (irritable bowel syndrome). These searches were meant to find studies on the safety and adverse events profile of FMTs in various clinical conditions. Upon reviewing the search results, a total of five publications were selected for being included in the present study, based on their relevance to the research in question. These publications comprised one article each on the topics of inflammatory bowel disease, recurrent Clostridiodes difficile infection and graft-versus-host disease, as well as two articles addressing irritable bowel syndrome. The significant fact is that all of the selected publications were published in the year 2023, which means that they meet the requirement that the study reflects the most up-to-date and relevant research in the field.

First Publication: FMT and GVHD

Graft-versus-host disease (GVHD) is a systemic immune reaction that can develop after a patient has received donor-derived stem cells. In GVHD, the immune cells from the transplant perceive the recipient´s tissues as unfamiliar and initiate an inflammatory response against them. This condition typically arises following allogeneic hematopoietic stem cell transplantation and most often has effects on organs like the skin, the GIT and the liver.(73,74)

The first publication on FMT and GVHD analyzed a total of 22 publications encompassing 23 studies that met the predefined inclusion criteria. These studies collectively reported on 242 patients with GVHD after allo-HSCT who underwent FMT treatment. The route of administration varied: 34 patients received FMT via oral capsules, 9 via colonoscopy, 89 via nasoduodenal or gastroduodenal tube and 76 via nasogastric tube or enema using MAAT013.(75)

MaaT013 is a standardized, pooled, full-ecosystem microbiota biotherapeutic product specifically designed for FMT. It consists of a consortium of gut microbiota derived from healthy donors and is formulated as a liquid suspension for administration via nasogastric tube or enema. It is intended to restore microbiome diversity and immune homeostasis, particularly in patients with dysbiosis-related conditions such as GVHD.(76)

Donor relationships differed, with 21 recipients receiving FMT from related donors, while the remainders had unrelated donors. The median number of FMT procedures per patient was three, with a maximum of ten. The average volume of transplanted fecal suspension ranged between 200 and 250ml.

This analysis identified a range of AEs associated with FMT, including infections, nausea, vomiting, abdominal pain, diarrhea, constipation, abdominal distension, fever, anorexia, general malaise, sore throat and gastrointestinal bleeding resulting from invasive procedures.

Regarding infections, the majority were classified as unrelated to FMT. These included two cases of sepsis, one case of septic shock, one Norovirus infection and one Adenovirus infection.

Despite the severity of these infections, all five patients responded promptly to appropriate medical treatment and achieved full recovery.

Moreover, it should be emphasized that patients undergoing treatment for GVHD following HSCT typically receive systemic immunosuppressive therapy for a minimum of six months.(77) Due to that fact, most FMT recipients in this population are in a state of immunosuppression, which is associated with an increased risk of gut microbiota translocation and subsequent infection.(78–80) Other adverse events previously mentioned were characterized in the publication as mild and transient in nature, with symptoms generally resolving spontaneously or with minimal medical intervention.

Furthermore, a total of 90 deaths were documented across the analyzed studies. However, none of these fatalities were attributed to FMT, suggesting that the procedure itself was not a contributing factor to mortality in the studied patient population.

The publication highlighted the fact that the choice of donor, whether related or unrelated, did not have any significant impact on the safety outcomes associated with the procedure. This suggests that both related and unrelated donors can be considered equally viable in terms of safety for FMTs in the treatment of GVHD.(75)

Second Publication: FMT and irritable bowel syndrome

IBS is a chronic gastrointestinal condition characterized by abdominal discomfort linked to altered bowel habits, such as changes in stool consistency or frequency. It affects approximately 5% to 10% of the general population at any given time. The clinical course of IBS is typically marked by alternating periods of symptom flare-ups and remission and while the disorder does not cause structural damage, it can significantly impact quality of life.(81,82)

First publication on FMT and irritable bowel syndrome

The first study included eight randomized controlled trials (RCTs) comparing FMT to placebo for the treatment of irritable bowel syndrome (IBS), irrespective of the study population characteristics or language of publication, comprising a total of 484 participants.

Due to the lack of standardized protocols for FMT, the procedures varied substantially across studies. Trials were included regardless of the quantity of fecal material used, the form of the material (fresh or frozen), the route of administration, treatment frequency (single versus multiple infusions) and donor selection (related or unrelated).

Three of the included trials administered FMT via colonoscopy, one via gastroscopy, one via nasojejunal tube and three through oral capsules.

The volume of the transplanted material ranged from 100ml to 300ml, while the quantity of fecal matter used varied between 30 and 80 grams.

Six trials implemented bowel cleansing prior to FMT administration and two trials administered loperamide beforehand to enhance retention of the transplant. One trial used proton pump inhibitors for three days prior to transplantation.

The proportion of patients experiencing non-serious and serious adverse events, as well as dropout rates due to adverse events, were defined as secondary outcomes. These were assessed at both three and six months following the intervention.

Data from seven RTCs involving 450 participants revealed that adverse events were reported in 35% (97 out of 274) of individuals receiving FMT, compared to

26% (45 out of 176) in those assigned to placebo. The majority of these adverse events were characterized by mild and short-lived gastrointestinal disturbances. Serious adverse events were documented across all eight included studies, encompassing a total of 501 participants. Within the FMT cohort, one participant (out of 302) experienced a serious adverse event, whereas two such events (out of 199) were noted among placebo recipients. Notably, one placebo participant died by suicide 10 days following the transplantation procedure. In addition, another participant of the placebo group required hospitalization for acute cholecystitis in week 20 of the study. Furthermore, one participant from the FMT group was hospitalized post-procedure due to transient vertigo accompanied by nausea.

Across eight trials with 502 participants, no dropouts occurred in the FMT groups while two dropouts were reported in the placebo groups due to adverse events. One placebo participant withdrew because of post-procedure discomfort and another dropout was related to the previously reported suicide 10 days after transplantation.(83)

Second publication on FMT and irritable bowel syndrome

The second study reviewed randomized controlled trials involving IBS patients diagnosed using Rome III or Rome IV criteria. It included FMT interventions regardless of administration route or dosage and compared outcomes against placebo groups.

Seven systematic reviews published between 2019 and 2023 were analyzed.(84) The Rome criteria mentioned above provide standardized symptom-based guidelines for diagnosing functional gastrointestinal disorders like IBS in the absence of organic disease. They ensure consistent diagnosis, support clinical management and facilitate research comparability.(85)

Although the authors emphasize that current evidence is insufficient to confirm the effectiveness of FMT for treating IBS and call for more robust trials – particularly regarding donor selection, dosage and administration – they still report that five of the included systematic reviews consistently found no significant difference in adverse event rates between FMT and placebo groups.(84) This suggests that, while efficacy remains uncertain, the safety profile appears comparable.

Fourth publication: FMT and inflammatory bowel disease

Inflammatory bowel disease (IBD) refers to chronic, immune-mediated inflammation of the GIT, probably driven by an abnormal response to intestinal microbiota. It includes two primary conditions: ulcerative colitis and Crohn´s disease. The two diseases differ in both location and depth of tissue involvement. UC causes superficial inflammation confined to the colon, often beginning in the rectum and potentially extending throughout the colon.
CD, in contrast, involves deeper, transmural inflammation that can affect any part of the GIT, most commonly the terminal ileum and colon.(86,87)
This comprehensive review examining the association between FMT and inflammatory bowel disease incorporates data from twelve RCTs encompassing a total of 550 participants. Studies considered for inclusion took up participants diagnosed with ulcerative colitis (UC) or Chron´s disease (CD) through clinical history, physical assessment, as well as macroscopic endoscopic and histopathological findings.
All included studies utilized stool samples obtained from apparently healthy donors. In nine studies, donors were unrelated to recipients, whereas one study exclusively involved related donor-recipient pairs, and another one employed a combination of related and unrelated donors. One study did not explicitly report on the donor-recipient relationship.
Regarding the route of administration, two studies delivered FMT exclusively via the upper GIT, using either oral capsules or nasoduodenal tubes. Nine studies administered FMT via the lower GIT, primarily through enemas or colonoscopy. One study combined oral capsule delivery with colonoscopic administration.
The frequency of FMT administration varied considerably. Two studies employed a single dose, while others administered multiple doses, with a maximum of 85 treatments reported.
The stool mass used per FMT ranged from 0.35 grams (per capsule) to 120 grams (via nasoduodenal tube) and the administered volume varied between 50ml and 500ml per session.
Colon preparation was performed prior to FMT in nine studies, whereas the remaining studies did not utilize bowel cleansing protocols.

In this review, serious adverse events in UC or CD, as defined by the study authors, were considered as primary outcomes, while any adverse events in UC or CD, also defined by the study authors, were counted as secondary outcomes. The review categorizes the data into four distinct subgroups:

1. FMT versus control for induction of remission in UC
2. FMT versus control for maintenance of remission in UC
3. FMT versus control for induction of remission in CD
4. FMT versus control for maintenance of remission in CD

First subgroup

In the first subgroup data about SAEs were reported in ten studies evaluating FMT for induction of remission in patients with UC. However, the certainty of evidence regarding the risk of SAEs associated with FMT in active UC was very low, primarily due to potential bias in the studies. In the FMT group, 22 out of 224 participants experienced SAEs, compared to twelve out of 224 in the placebo group. Reported SAEs included exacerbation of UC requiring corticosteroid treatment or surgery, infections, small bowel perforation and pneumonia. Regarding any AEs, there was little to no difference observed between the FMT and control groups, with 96 of 208 participants in the FMT group and 95 of 209 participants in the control group reporting AEs. The most commonly reported AEs were abdominal pain, nausea, flatulence, bloating, headaches and dizziness.

Second subgroup

In the second subgroup, ten studies reported no SAEs among 71 UC patients who received FMT for maintenance of remission. The certainty of evidence regarding SAE risk in this context was also deemed uncertain.
Concerning any AEs, data from two studies provided very uncertain evidence about the risk associated with FMT for maintenance of remission in UC. In these studies, 22 of 35 participants in the FMT group experienced AEs compared to eight of 36 in the control group.

Third subgroup

Within the third subgroup, the review found that none of the analyzed studies presented evidence or reported outcomes related to the use of FMT for the purpose of inducing remission in patients diagnosed with CD.

Fourth subgroup

The final subgroup, labeled as FMT for maintenance of remission in CD, included a single study of 21 participants that reported a total of 13 SAEs. However, the study did not clarify how these events were distributed between FMT-treated and control groups.

Similarly, for general AEs, this study mentioned their occurrence but failed to provide the overall count or to offer data on how they were allocated across the two groups. Furthermore, it was not specified whether the reported events corresponded to individual patients or if some participants experienced multiple AEs.(88)

Fifth publication: FMT and recurrent Clostridioides difficile infection

Clostridioides difficile infection is the leading cause of antibiotic-associated diarrhea. Following successful treatment, recurrent CDI frequently develops, posing significant challenges for management. The rising prevalence of rCDI and its treatment complexity have made it a growing concern in clinical practice.(89–92)

FMT has demonstrated promising results in the management of rCDI.(93)

The final systematic review assessed the effectiveness of FMT in managing recurrent rCDI. Individuals were considered eligible if they experienced frequent or watery diarrhea alongside diagnostic confirmation of Clostridioides difficile, either via stool testing or characteristic colonoscopy/histology findings. Recurrent CDI was defined as a return of symptoms after successful initial antibiotic treatment – such as with vancomycin, fidaxomicin, or metronidazole – paired with a renewed positive test result.

In the reviewed study SAEs, as defined by the study authors, were described as a primary outcome, while the occurrence of any AEs was listed as a secondary outcome.

The review included six randomized controlled trials evaluating FMT for rCDI infection. Five of these excluded immunocompromised patients, while one trial enrolled ten participants on immunosuppressive therapy out of a total of 64, with similar distribution between the FMT and control groups.

All studies used stool from apparently healthy donors. In half of the trials, donors were unrelated to recipients, while in the others, both related and unrelated donors were included. Each FMT used stool from a single donor rather than pooled samples.

Regarding delivery methods, one study administered FMT via nasoduodenal tube, another one used either nasojejunal or colonoscopic routes. Two used enemas and two used colonoscopies alone.

Three trials employed a single FMT administration, while the remaining three allowed multiple treatments.

The amount of stool used per FMT procedure varied from 50 grams to a mean of 152 grams, while the volume administered ranged between 170 and 500 ml. One study did not report the volume used.

As part of the preparation protocol, five of the six studies included colonic lavage prior to FMT administration. The remaining study omitted this step.
Follow-up durations for assessing the primary outcome ranged from eight to 17 weeks.
The review compiled data from six RCTs involving 320 participants – 133 received FMT treatment and 187 were in the control groups.
All studies reported SAEs, with 18 events in the FMT group and 42 in the control group.
Additionally, all six studies tracked any AEs. In the FMT group 111 individuals experienced 189 AEs, while in the control group 163 participants reported 164 AEs.
Most AEs described in the FMT group were mild and of gastrointestinal nature, such as abdominal pain, bloating and diarrhea.(94)

Data researched at the Medical University of Graz

In addition to the carried-out literature research comprehensive data from FMT procedures conducted at the Medical University of Graz was systematically entered into an official registry maintained by the Austrian Society of Gastroenterology and Hepatology (ÖGGH). This data was then thouroughly analyzed to evaluate the outcomes and implications of these procedures. The FMTs recorded in the registry were performed for a variety of clinical conditions, including ulcerative colitis, irritable bowel syndrome, metabolic syndrome, and graft-versus-host disease.

Subjects in the cohort

A total of 118 subjects were included in the study. All of the screening was done according to an internally set up guideline. An analysis of baseline demographic characteristics revealed that the median age of the patient population at the time of intervention procedure was 45 years. All participants underwent a standardized colon lavage procedure. In addition, comprehensive screening was conducted in accordance with internal guidelines to ensure eligibility. Following this screening process, all subjects were confirmed as suitable and were actively participating in the study. Of the enrolled participants, 50,8% were male, while the remaining 49,2% were female.

Each participant underwent an FMT application administered to the lower gastrointestinal tract via colonoscopy.

It is important to mention that both fresh and frozen stool samples were being employed. In cases where frozen stool was used, it was stored at -80 degrees Celsius for an average of 13 weeks prior to administration. A total of 44,1% of patients underwent treatment with frozen stool, whereas 54,2% received fresh stool preparations. For 1,7 % of the cases, data on the type of stool application was unavailable.

The administration was solely performed via the large intestine. Specifically, 78% of the patients received the application through the right colon, while 8,5%

underwent administration via the left colon. For 13,6% of cases, data regarding the exact site of application was missing.
All primary FMT procedures were conducted in an inpatient setting. Variability was observed in the frequency of stool transplants administered. Repeated transplantations were performed in the outpatient clinic setting. Detailed information on the frequency of single versus multiple FMTs is provided in the table below.

Single FMT	4,2%
Multiple FMTs	94,9%
Missing data	0,8%

Table 4: Frequency of single versus multiple FMT

For cases involving multiple FMTs, the minimum number of procedures was two, while the maximum number reached eight. The mean number of FMTs, which were performed, was three, reflecting the central tendency within the cohort of patients who underwent multiple transplants.

Indications for FMT in the cohort

As previously outlined, patients with a range of medical indications were treated with FMTs. Multiple pie charts display the distribution of the various indications observed within the cohort.

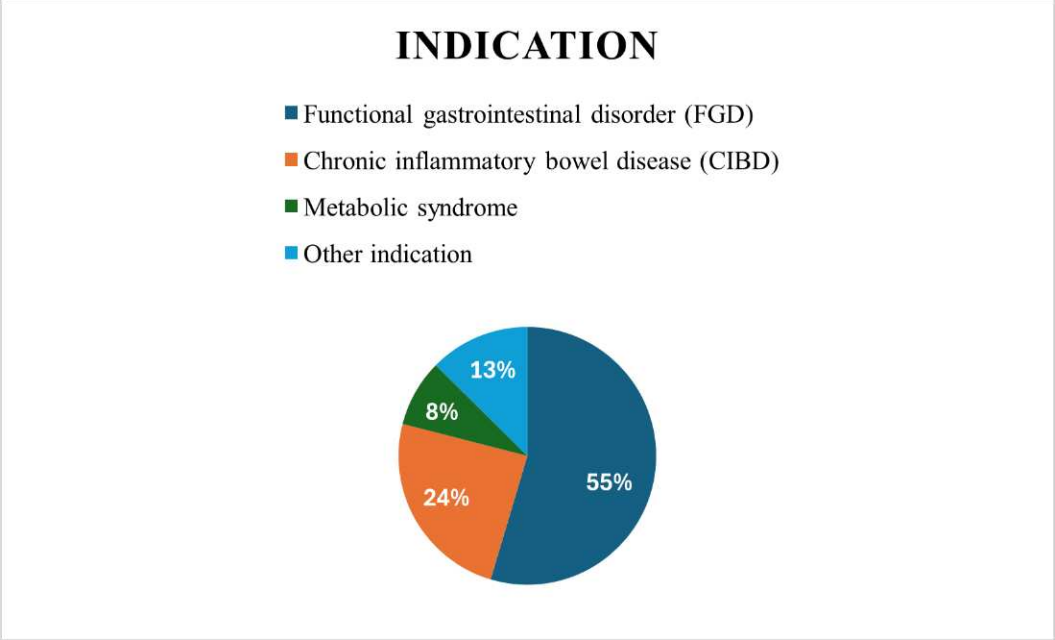

Figure 3: Indications for FMT in the cohort

The graph displays the distribution of indications observed in the cohort, categorized into four main conditions.
Functional Gastrointestinal Disorders (FGD) represent the majority, with 55.1% of individuals falling into this group. Patients in the group suffered from irritable bowel syndrome, a complex syndrome with varying symptoms such as abdominal pain, diarrhea or constipation, of which the exact cause is yet unclear.(95)
Chronic inflammatory bowel disease (CIBD), which includes Crohn´s disease and ulcerative colitis, affects 24.6% of the studied population and is characterized by persistent inflammation of the gastrointestinal tract.
Metabolic Syndrome was the reason for transplantation in 8,5% of individuals and generally includes a cluster of factors like obesity, high blood pressure or dyslipidemia, all of which contribute to increased cardiovascular risk.(96)

The remaining 12,7% of cases are categorized under “Other indications”, referring to various additional conditions that are not covered in the primary categories such as graft-versus-host disease.

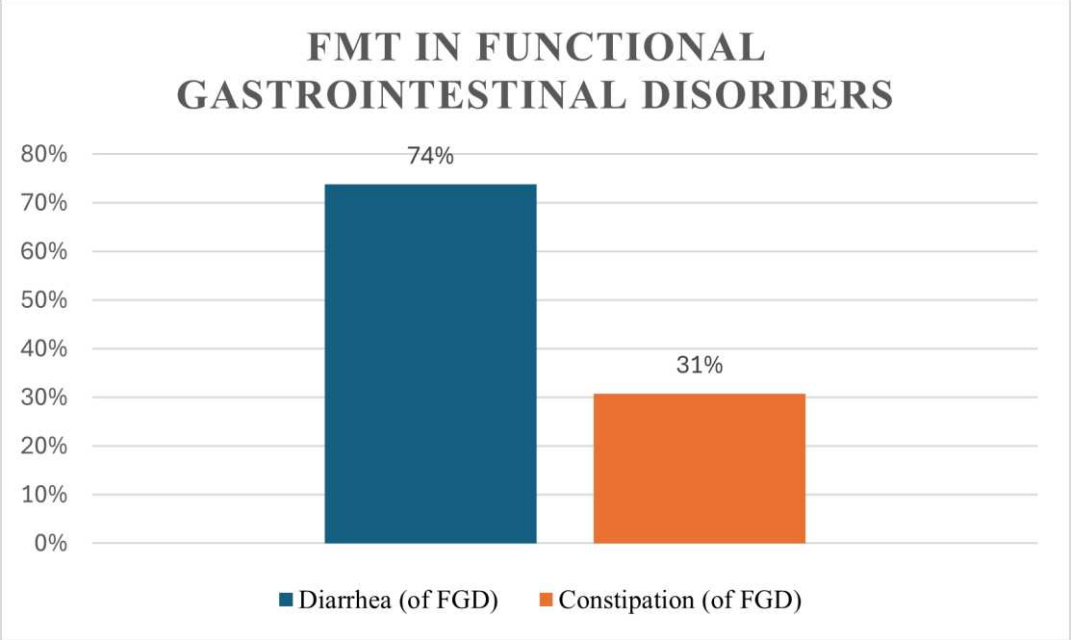

Figure 4: FMT in functional gastrointestinal disorders

Concerning FGDs 74% of the patients suffered from diarrhea whereas 31% were affected by constipation. Due to the fact that some patients had a mixed IBS-subtype with diarrhea as well as constipation, the total percentage in this figure exceeds 100%.

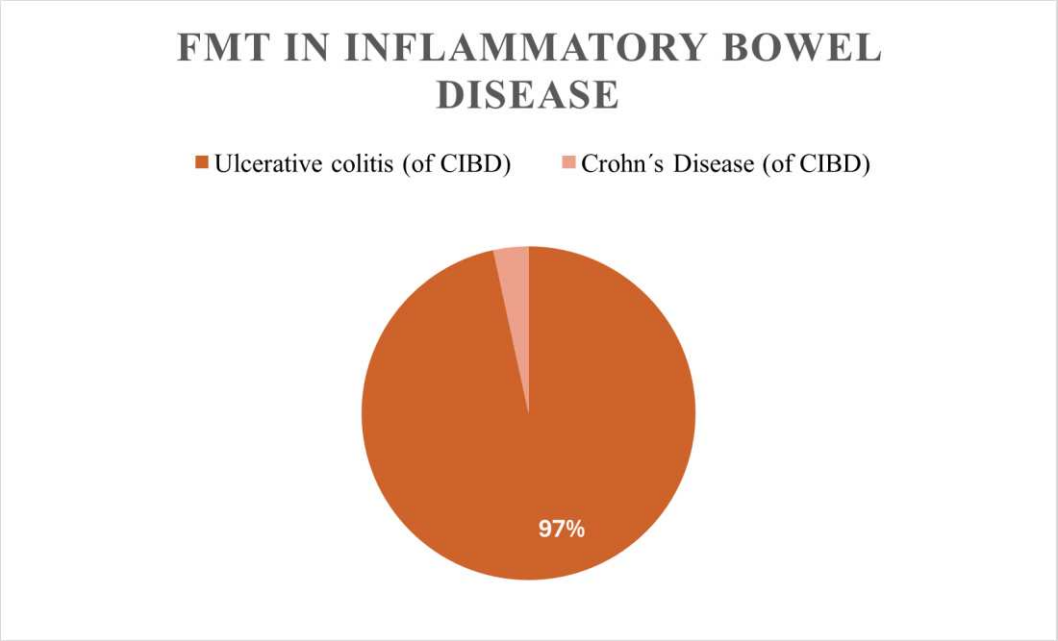

Figure 5: FMT in inflammatory bowel disease

The CIBD group consists of 97% ulcerative colitis and 3% Crohn´s disease, which is illustrated in the pie chart above.

Secondary conditions in the cohort

Alongside primary diagnoses, secondary conditions were systematically recorded for all subjects. In an analysis of comorbidities within this patient cohort it was found that 59,30% of the patients exhibited no relevant comorbid conditions. This finding suggests that the majority of individuals in the studied population is probably in a relatively good state of health.
Only 1,70% of patients were reported to have coronary artery disease. Furthermore, no patients were documented as experiencing respiratory insufficiency, liver insufficiency, or kidney insufficiency. This absence supports the notion that the patients generally maintain well-functioning organs and systems, apart from their problems requiring FMT- treatment.
However, it is worth noting that 17,80% of patients were classified as having unspecified comorbidities. This points to the potential for additionally underlying health issues that were not explicitly categorized within the standard classifications utilized in this analysis.

In conclusion, when considering the aggregation of secondary conditions within this patient cohort, the findings indicate that a predominant majority of patients present themselves without any comorbidities. Conversely, only a limited number of individuals exhibit specific health issues.

Medications of patients in the cohort

In the context of this study it was observed that the population was required to take a variety of medication regimes that were maintained independent of their FMT treatment.
Among the cohort 22% of the patients were not receiving any form of pharmacological intervention, indicating that a subset of individuals had no medical indications for treatment.
Additionally, 3,4% of patients were prescribed proton pump inhibitors (PPIs), which are commonly utilized for the management of gastric acid-related disorders.(97,98)
Moreover, immunosuppressive therapy was identified in 23,7% of the patient group, highlighting the presence of underlying conditions that may necessitate such treatment.
The remaining 64,4% of patients were classified under other medication categories, reflecting a diverse range of therapeutic approaches tailored to their specific clinical needs.

Donors of the Cohort

The BMI of the donors ranged between a minimum value of 19 and a maximum of 29. The median BMI was calculated to be 22.
In addition to BMI, an analysis of the demographic characteristics of the stool donors revealed that the median age of the donors at the time of the stool donation was 35 years.
All stool donors were systemically and rigorously screened in accordance with the internally established guidelines as described in the example analyzed in the chapter “Donor selection”. This comprehensive screening process ensured that each donor met the stringent criteria required for inclusion in the study, thereby minimizing potential risks and ensuring the quality and safety of the transplants carried out on the recipient patients.

Relationship between Donors and Recipients

Regarding the relationship between donors and recipients, 10,2% of the donors were genetically related to the recipients.
Moreover, 6,8% of the donors were not related, however, known to the recipients and resided in the same household, while 2,5% were known but did not live in the same household.
The majority, 61% of the donors, were external donors organized by the study team who therefore were not personally acquainted with the recipients.
Data on the relationship between the donor and recipient were missing for 19,5% of cases, suggesting a notable gap in the available information.
An overview of the relationships between recipients and donors can be seen in the figure below.

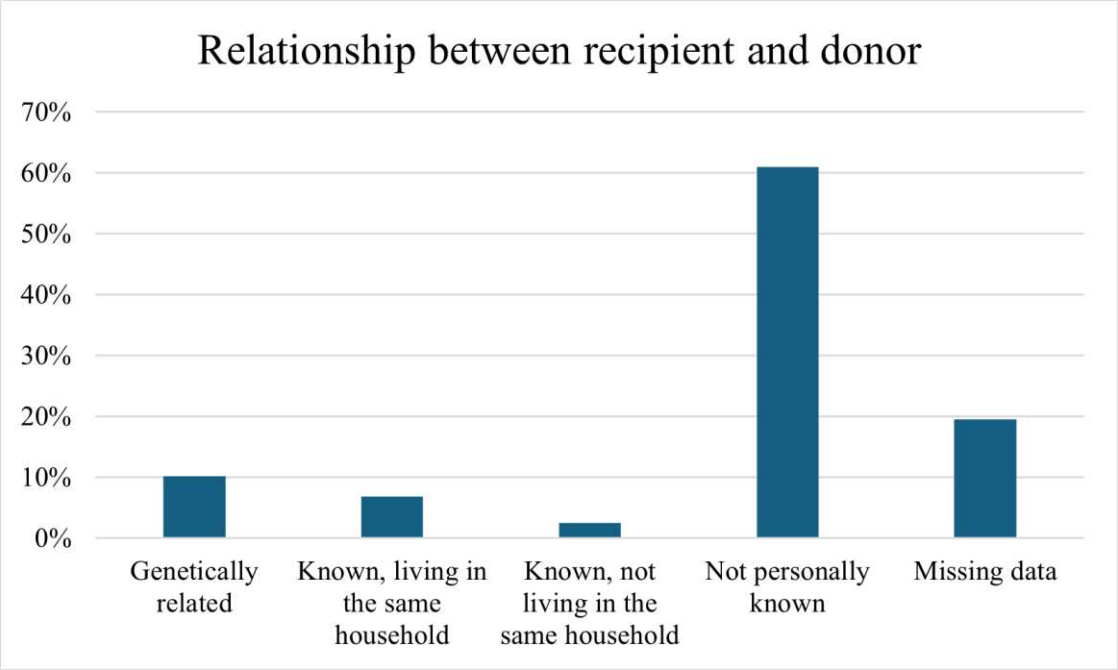

Figure 6: Relationship between recipient and donor

Post FMT Effects

Adverse effects were comprehensively assessed in the patient population to address the central question of the study. All of the following effects occurred immediately after the FMT.

It is crucial to acknowledge that these adverse effects may not solely result from the FMT itself but could also be attributable to the preceding colonoscopy procedure.

Among the patients evaluated, a significant proportion (56,8%) reported no aftereffects subsequent to the treatment, indicating a favorable outcome for a majority of individuals.

Conversely, fever was documented in 1,7% of the cohort, while symptoms like meteorism, characterized by abdominal distension, were observed in 6,8% of the patients.

Diarrhea was reported in 5,9% of cases, and 11% of the patients experienced varying degrees of pain.

Additionally, 5,9% of the patient population noted a deterioration in their preexisting medical conditions.

Other unspecified adverse effects were reported in 11,9% of individuals.

To facilitate a clearer understanding of the distribution of these adverse effects and their relative frequencies, a diagram illustrating these findings is provided below.

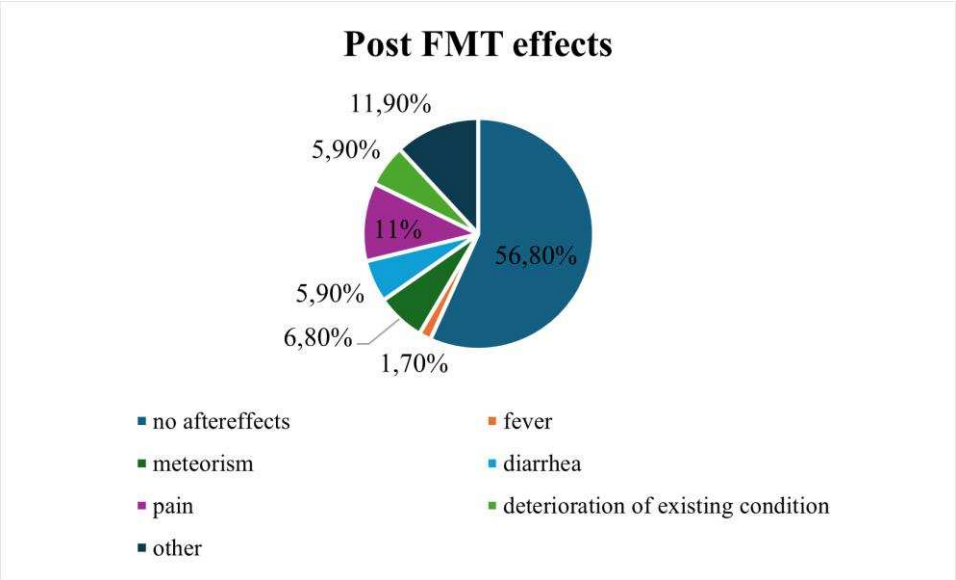

Figure 7: Post FMT effects

BMI changes in the cohort

The Body Mass Index (BMI) of the recipients was measured at two different points: first before the FMT procedure and then again during the follow-up period. This measurement is especially significant for patients diagnosed with metabolic syndrome, as it provides valuable insights into changes in BMI related to the condition and the intervention.

The graph provided below illustrates the BMI values presented as minimum, maximum, median and mean values. These measurements are reported both prior to (blue) and following fecal microbiota transplantation (orange).

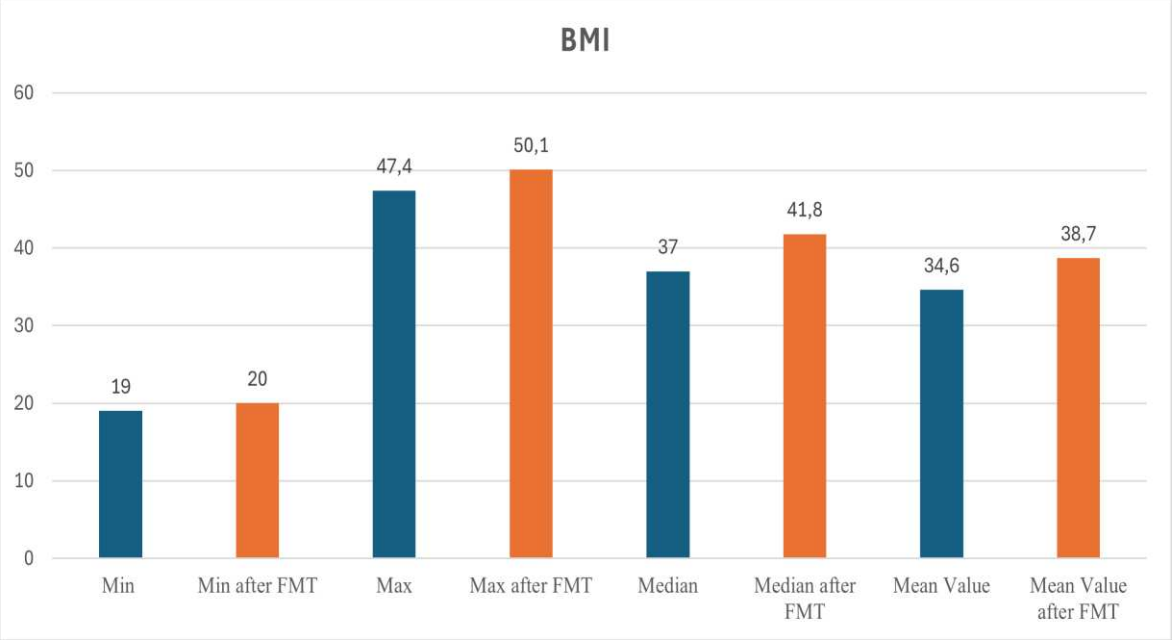

Figure 8: BMI changes in the cohort

The detailed examination of the BMI values before and after FMT treatment reveals noteworthy alterations in the weight status of the patient cohort.

Initially, the minimum BMI prior to FMT was 19. Following the procedure, this minimum value increased to 20.

Before FMT, the maximum recorded BMI was 47,4, categorizing this patient as obese. Post-intervention, the maximum BMI rose up to 50,1.

The median BMI also exhibits a marked upward trend. Initially, the median BMI was 37, reflecting a central tendency towards obesity within the cohort. After FMT,

this median value increased to 41,8, signifying an overall rise in body weight among the patient group subsequent to the intervention.
Similarly, the mean BMI illustrates a comparable upward shift. The average BMI prior to FMT was 34,6, situating the population within the overweight to obese range. Following the procedure, the mean BMI went up to 38,7, a further proof of the observation that the average body weight among patients increases after FMT.

Follow-up period

Among the cases observed, a total of 57 valid cases were documented during the follow-up period. The time interval between the follow-up assessment and the initial examination had a minimum of 37 days. Among these patients, 45,8% did not receive any antibiotic treatment during the interim period, whereas 0,8% did undergo antibiotic treatment. However, for the remaining 53,4% of cases, data on antibiotic administration was either incomplete or unavailable, making it impossible to draw definitive conclusions regarding their antibiotic exposure during the follow-up period.
Among the patients who underwent an FMT, an overwhelming 96,5% showed no evidence of new diseases during the follow-up period, suggesting a favorable outcome with regard to the development of new health conditions.
However, data for the remaining cases was either incomplete or not available. Interestingly, all other categories of new diseases, which included gastrointestinal disorders, metabolic conditions, neuropsychiatric disorders, autoimmune diseases, malignancies, and “other new diseases”, were reported at a rate of 0%, meaning that no new occurrences of these conditions were observed within the cohort throughout the follow-up period. This is visually represented in the graph below.

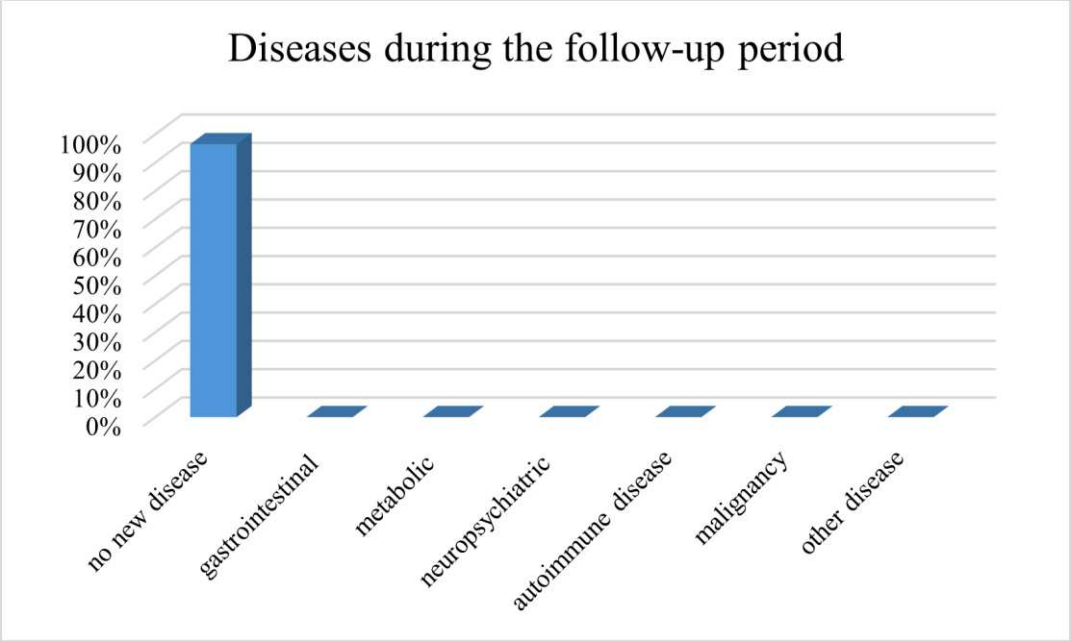

Figure 9: Diseases during the follow-up period

Among the patients analyzed, 98,2% were documented as alive at the time of evaluation. Though, it is important to note that no definitive conclusions can be drawn regarding the remaining cases, as the data for these individuals is incomplete or unavailable. A more detailed examination of the data-set further reveals that none of the patients was mentioned in the “deceased – yes” category, indicating that no confirmed fatalities were reported within the available data.

Discussion

Overview of the data collected at the Medical University of Graz

This study, which was conducted at the Medical University of Graz, included a total of 118 participants at a median age of 45 years and a nearly equal gender distribution. FMT was administered exclusively via colonoscopy, with 78% of applications performed in the right colon. The majority of patients received multiple transplants, as repeated FMT may be necessary to achieve sustained clinical benefits.

Both fresh and frozen stool preparations were used, although missing data of some cases present a limitation.

The most common indication was irritable bowel syndrome. Chronic inflammatory bowel disease accounted for nearly a quarter of cases, predominantly ulcerative colitis.

The majority of patients had no relevant comorbidities, indicating a relatively healthy study population, apart from their underlying disease, and thus limiting generalizability to more complex clinical cohorts.

Post-FMT symptoms were generally mild and short-term, with over half of the patients reporting no complications. Whether the symptoms were caused by FMT or colonoscopy remains unclear. There were no reports for AEs or SAEs in the follow-up period.

BMI measurements indicated a general increase post-FMT, suggesting potential metabolic impacts. This fact is remarkable and definitely needs to be considered when applying FMT treatment. The trend towards higher body weight status may have significant implications for the metabolic health and overall well-being of the patients involved in this study, justifying further investigation into the underlying mechanisms and long-term effects of FMT on weight dynamics. One possible explanation for the observed increase in BMI is that approximately 25% of the patients who received FMT had inflammatory bowel disease, specifically ulcerative colitis. Active inflammation in UC is often associated with weight loss and a lower BMI. Since FMT has been shown to effectively reduce intestinal inflammation in

UC, clinical improvement may allow these patients to regain weight, contributing to the overall trend toward higher BMI.

Overview of literature on FMT safety

In addition to the data collected at the Medical University of Graz, five studies on adverse events related to FMT were reviewed. Search terms included combinations of FMT with “safety” and either “inflammatory bowel disease”, “recurrent Clostridioides”, “graft-versus-host disease” or “irritable bowel syndrome”. For each topic one study was analyzed, except for IBS, for which two studies were surveyed.

FMT and graft-versus-host disease

The GVHD-related study classified a range of adverse events, including infections, gastrointestinal symptoms and procedure-related bleeding. However, most infections which occurred were considered unrelated to FMT. Other events were described as mild and transient, resolving spontaneously or with minimal intervention.

FMT and irritable bowel syndrome

In the first IBS study, adverse events were reported in 35% of FMT recipients compared to 26% in a placebo group, with most events being mild, short-lived gastrointestinal disturbances. Serious adverse events occurred in one FMT recipient and in two placebo recipients, including one suicide and one case of acute cholecystitis requiring hospitalization. Additionally, one recipient was hospitalized due to transient vertigo with nausea.

The second IBS study concluded that current evidence is insufficient to confirm the efficacy of FMT for IBS and called for more rigorous trials. However, five reviews included in this study reported no significant differences in adverse event rates between FMT and placebo groups.

FMT and inflammatory bowel disease

The IBD study evaluated adverse events in four subgroups:
The evidence resulting from the groups is characterized by low certainty. Most reported AEs were mild and self-limiting. SAEs occurred rarely, however, were inconsistently reported across all four groups.

FMT and recurrent Clostridioides infection

The safety profile of FMT in rCDI was evaluated in a review comprising six randomized controlled trials involving 320 participants. Of these, 133 received FMT whereas 187 were assigned to control groups. All studies reported serious adverse events, with 18 events occurring in the FMT group compared to 42 in controls. Additionally, 111 FMT recipients experienced 189 adverse events, while 163 control participants reported 164 adverse events. Most AEs in the FMT group were mild and gastrointestinal in nature, including abdominal pain, bloating and diarrhea.

Limitations

The limitations of this study are significant and must be carefully considered when interpreting the results.
Firstly, the retrospective design, used in this study referring to the data from the Medical University of Graz, inherently limits the quality and completeness of the facts collected. As is often the case with retrospective analyses, essential information may be missing, inconsistently documented or affected by bias due to non-standardized data collection. These issues are reflected in several aspects of this thesis, including missing values related to the route of administration and stool preparation type, which might affect the accuracy of subgroup analyses and overall conclusions.
Secondly, a major challenge in evaluating FMT studies in general lies in the lack of standardized protocols. Variables such as donor selection criteria, route and site of administration, stool volume, consistency and the number and timing of FMT

applications vary widely across studies and even within single cohorts. This heterogeneity makes it almost impossible to define large, uniform patient groups for reliable statistical comparisons.
Consequently, drawing definitive conclusions about the efficacy or safety of FMT in specific subgroups remains problematic.
In the present study, this issue is compounded by the relatively small sample size of the cohort at the Medical University of Graz, which limits statistical power and the ability to detect subtle effects or rare AEs, respectively SAEs.
Many of the other studies cited had larger participant groups, increasing their statistical power to observe less common effects and thus bolstering the confidence in their conclusions.
Another critical limitation is the limited reporting on adverse events. Most available data, including this study, focuses on short-term safety outcomes, with little to no information on long-term or delayed AEs and SAEs. While reported short-term AEs appear to be infrequent and generally mild, many studies – including this one and the ones cited – acknowledge a degree of uncertainty due to incomplete follow-up, underreporting or a lack of standardized monitoring protocols. This makes it difficult to assess the true risk profile of FMT, especially when applied in broader or more vulnerable populations.
Additionally, the inclusion of immunosuppressed patients complicated the interpretation of safety data even more. These individuals may be at a higher risk of infections or other complications following FMT, yet many studies in general do not classify outcomes based on immune status or provide sufficient data to evaluate differential risks.
Another limitation of the data from the Medical University of Graz is the fact that there was a control group for only two indications, namely for functional GI disorders and obesity, for the other indications like UC or GvHD there were no control groups available.
As the present work focuses on AEs and SAEs the data of the control groups have not been analyzed. Without a placebo or standard treatment comparator, it is challenging to determine the extent to which observed effects can be attributed directly to FMT versus natural disease progression or other confounding factors.
In contrast to the data of the Medical University of Graz, all of the previously cited

studies included data of control groups, which enhances the reliability of their findings.

In summary, the retrospective nature of the study, the lack of protocol standardization, the incomplete data and the limited insight into long-term safety outcomes all represent significant limitations. Future prospective studies with standardized methodologies and longer follow-up periods are essential to improve the reliability of FMT research and to better understand its risk-benefit profile among diverse patient populations.

Conclusion

The primary objective of this thesis was to investigate the safety of Fecal Microbiota Transplantation, focusing specifically on adverse events and serious adverse events. To achieve this, a retrospective analysis of patient data from the Medical University of Graz was conducted alongside a targeted review of selected studies reporting on FMTs in various diseases.

The retrospective data from Graz proved difficult to evaluate due to limited and inconsistent documentation, making it challenging to draw robust conclusions. Similarly, the evidence from the literature – although broader in scope – was frequently marked by low certainty, small sample sizes or incomplete reporting. Nevertheless, in the available studies, FMT was generally well tolerated with only mild and transient gastrointestinal symptoms. Serious adverse events were rare but did occur, especially in vulnerable patient populations. Thus, careful monitoring is required.

Overall, while Fecal Microbiota Transplantation appears to be generally safe across various clinical indications, the available evidence is often limited by low certainty and incomplete reporting of adverse events.

To establish clearer safety standards, future research is to focus on well-designed, large-scale studies with consistent reporting frameworks. In addition, standardized protocols regarding donor selection, preparation, administration method and long-term follow-up will be essential to ensure both the efficacy and the safety of FMT in clinical practice.

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