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Fecal Transplantation

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ABBREVIATIONS

FT	Fecal transplantation
CDI	Clostridioides difficile infection
C. difficile	Clostridioides difficile
FDA	American Food and Drug Administration
IV	Intravenous
IBD	Inflammatory bowel disease
UC	Ulcerative Colitis
CD	Crohn's disease
GI	Gastrointestinal
ASD	autism spectrum disorder
GSRS	Gastrointestinal symptom rating scale
5-HT	5-Hydroxytryptamin
GABA	γ- aminobutyric acid
DA	Dopamine
HbA1c	Glycated hemoglobin type A1c
IL-25	Interleukin

ABSTRACT

A dysbiosis of the intestinal microbiome is a major etiological factor in the pathogenesis of many different diseases. Especially in *Clostridioides difficile* infection, the imbalance is very prominent, but also in Inflammatory Bowel diseases, Psychiatric diseases, Metabolic syndrome, and malignancies it can be observed. One way of manipulating the composition of the gut microbiome is Fecal transplantation, which has become a routine treatment method for recurrent/refractory *Clostridioides difficile* infection and has proven to be more effective and at a reduced cost compared to antibiotic treatment. The different methods of application are Colonoscopy, Oral Capsules, Enema and Nasogastric tube, that all proved to be very effective, but Oral capsules are especially convenient for the patient and the health care provider. Regarding the side effects, fecal transplantation has also been confirmed to be safe. Also in Inflammatory Bowel diseases, several trials have been conducted and a disease remission could be observed, yet fecal transplantation for the treatment of Ulcerative Colitis showed more promising results than it did for Crohn's disease. A depletion in gut microbiota has also been found in depressed patients and children with autism spectrum disorder. Fecal transplantation improved both gastrointestinal and autism spectrum disorder symptoms in these patients. Furthermore, also in patients with metabolic syndrome changes in the microbiota composition have been observed, though controversial results in the efficacy of fecal transplantation for the improvement of insulin sensitivity were detected. In metastatic melanoma patients that were unresponsive to a type of immunotherapy, received a fecal transplant from responders and as a result an advance in the response to the treatment was noted. Additionally, fecal transplantation could be a potential treatment option for chemotherapy associated gastrointestinal patients even in immunosuppressed individuals. The exact mechanism of action of fecal transplantation is still unknown, though several hypotheses have been suggested. Post fecal transplantation, an increase in butyrate-producing bacteria and an enhancement of secondary bile acid metabolism were found that both have a beneficial effect on the defense against imbalances and pathogens. Furthermore, niche exclusion and immunologic pathways are also a part of the mechanism of action of fecal transplantation. It is important to note that human stool is comprised of other parts but bacteria, that possibly also contribute to the pathophysiologic mechanism of the procedure.

Keywords: Fecal transplantation, Human microbiome, Clostridioides difficile infection, Gastrointestinal microbial disbalance, Gut-brain axis

INTRODUCTION

Within the last decades, the human gut microbiome has received increasing attention for its outstanding role in optimal health and is seen as a hotspot in research. Not only have the numbers of publications increased but their contents have gradually become more specific and precise (1). In humans, the gut microbiome is thought to be home of several trillion microorganisms, mainly bacteria, but also viruses, fungi and single cell parasites. Out of all organs, the large intestine has the highest density of microbes namely up to 10^{11} cells per millilitre of intestinal contents and modern techniques have identified around 500-1000 different species. While every human has its individual unique microbiome, certain functional groups remain the same. Not only are the gut microbiota responsible for digestion of food, but also for the production of nutrients like biotin, Vitamin K and short chain fatty acids and they also aid in the fight against possible pathogens (2). Nowadays, the interest in the human gut is not mainly limited to infectious diseases anymore, but disbalances of intestinal microbiota are associated with obesity (3), cardiovascular disease (4) and gut microbes are even thought to influence the gut-brain axis communication (5), though overinterpretation of these results should be avoided on the grounds of extensive ongoing research. Due to the aforementioned complexity and the vast interactions and functions of the human gut, a disbalance of the gut microbiota can be of serious consequences for the host. Especially antibiotic treatments are responsible for a decrease in gut bacterial diversity and are a possible risk factor for off balance overgrowth of certain potentially harmful bacteria (6). Different treatment options to alter the microbiome include antibiotics, probiotics, special diets, lifestyle modifications, certain drugs and the within the last two decades increasingly popular faecal transplantation (7). In some sources it is termed Fecal microbial transplantation, but fecal transplantation, as used in this analysis, would be the more suitable term, since not only microbiota but also metabolites are being transplanted. In FT the stool of a healthy donor with its microorganisms is transferred to a sick recipient with the goal to restore the normal microbiota in the gut that currently is out of balance and leading to a disease and complaints.

LITERATURE SEARCH STRATEGY

Since FT is a rather new procedure, most sources used for the drafting of this paper are from the years 2013 to 2022 with a few exceptions. The main database used to search for suitable clinical trials was pubmed. As FT is such a novel treatment, and only routinely used in the treatment of

recurrent or refractory CDI, it was difficult to find large scale clinical trials regarding other possible medical uses of the procedure and no guidelines are existing up to this date. Merely for the treatment of refractory CDI there is a European consensus on FT and both European and American guidelines on the treatment of CDI. Moreover, during the search, it became evident that Clostridium difficile had been renamed into *Clostridioides difficile* in 2016 and literature used before that still used the old termination. Furthermore, the pathophysiologic mechanism behind microbiota transplant is not yet clearly understood, thus this literature review highlighted the most likely hypotheses and connected them with up-to-date research.

HISTORY OF FT

Faecal transplantation is not merely a concept of the 21st century, even though it has become a popular treatment method in modern days. Already in the fourth century Ge Hong, a traditional Chinese medicine doctor, described in his book "*A Handbook of Formulas for Emergencies*" where he used human faecal suspensions for patients who were suffering from food poisoning or severe diarrhoea. Further records are found from the 16th century when the physician Li Shizhen described different recipes with a fermented faecal solution, fresh faecal suspension and dried faeces of adults and children that were used to treat abdominal diseases that are accompanied by diarrhoea, fever, pain and vomiting, that soon later found its way to Europe (8). Christian Franz Paullini, a German physician, described in his book "*Heylsame Dreck-Apotheke*" in 1697 how diarrheal diseases can be treated by the ingestion of faeces (9).Additionally, the use of fresh camel stool by German soldiers to treat dysentery during the Second world war was reported, who apparently copied that method from the native Bedouins (10). The first in modern medicine described FT was done by Ben Eiseman, Leader of Surgery at the Denver General Hospital in 1958, curing several patients with a severe pseudomembranous colitis using faecal enemas (11). Since 1981, faecal transplantation in order to treat *C. difficile* associated Enterocolitis was applied several times throughout the entire world, with little to no public discussion. The transplantation methods used were rectal enemas, jejunal probes, nasogastric probes, duodenal probes and coloscopies. The therapy response rate varied from 69% to 100% (12). FT attracted more attention in 2013 when the first randomized controlled trial for the treatment of recurrent CDI was published in the *"New England Journal of Medicine"* especially since it seems to be very easy to use and extremely efficient in treating recurrent *C. difficile* infection. The trial was stopped early when 81% of the participants treated with FT had a resolution of their symptoms making FT a remarkably more

effective treatment method for recurrent *C. difficile* infection than using the antibiotic vancomycin for its eradication (13).

EPIDEMIOLOGY OF FT

Since the use of the treatment method faecal microbiota transplantation has been gaining in popularity over the past decades, a survey published in July 2021 on the use of Faecal Microbiota Transplantation in Europe has been published. Different FT centres in Europe were asked to fill out a questionnaire regarding the procedure. Out of all the FT procedures performed in 17 different countries, 58% had CDI as an indication. Denmark had the highest number of FTs performed per 100.000 inhabitants for CDI, followed by two other participating Nordic countries namely Iceland and Finland (14). This might be partially related to the fact that it was a Nordic based study. When comparing the implementation of clinical trials worldwide regarding FT, most were registered in North America and China, followed by Western Europe. The main indications were *C. difficile* infections, Inflammatory bowel diseases or the gut-brain axis. Notably, most research has been conducted in high-income countries (15).

DONOR SELECTION AND STOOL BANKS

Choosing a healthy donor is essential for the success of FT. The *European consensus conference on faecal microbiota transplantation* recommends a thorough donor selection testing (16). A detailed anamnesis of the donors should be taken, clinical examination, blood and stool testing is necessary. The main objective of the donor selection process is the avoidance of possible transmission of an infection due to contaminated transferred faecal material. Possible risk factors should be identified and therefore a preferably written questionnaire asking for the medical history and habits should be filled out by the possible donor. In general, donors <60 years should be preferred in the selection process but people older than 60 years of age are not entirely prohibited from donating. Exclusion criteria are not limited to infectious diseases but also GI disorders, autoimmune diseases, and malignancy are included. Specific GI related requirements were added. Blood testing includes screening for especially transmittable diseases like viruses, but also full blood count, inflammatory parameters, electrolytes, liver, and kidney parameters are included. Furthermore, stool testing screens for enteric pathogens for instance *C. difficile* norovirus and certain protozoa but also for faecal occult blood are conduced prior to transplanting the stool. Currently no clear recipient selection criteria have been mentioned (16). No noteworthy advantage

of a related donor (patient-directed) over an unrelated donor (universal) has been found but some patients might feel more at ease and may be more likely to give their consent for the procedure if they know the source of the faecal material (17). Having to go through all of the testing before a single stool transplant procedure is not only very costly but also postpones the delivery of the treatment. If the first patient selected donor does not pass the testing procedure before the transplant, another donor must be found who also might not match the entirety of the strict requirements. Furthermore, different physicians might have different standards when it comes to screening the possible donors and their stool and processing it for the procedure. That is one of the reasons why instead of using a patient-selected donor, stool banking in a good option, especially to ensure a high quality of the specimen, enhance logistics and time management and minimize the risks for the recipient. Additionally, centralizing the stool donations also makes the treatment method of FT more widely available to a broader population and practitioners (18). One of the largest stool banks is OpenBiome (19). In 2018 the stool banks obtained 7536 stool donations from 210 donors and over 50% of these donations were turned into FT preparations (20). Evidently, one donor donates stool several times, which further facilitates the screening process.

INDICATIONS FOR FT

Currently, FT is mainly used for the treatment of *C. difficile* infection. It is recommended to use FT in refractory severe CDI in the 2021 update on the treatment guidance document for *Clostridioides difficile* infection in adults by the *European Society of Clinical Microbiology and Infectious Diseases* (21). It is important to state, that these conventions cannot readily be applied to other diseases. Inflammatory bowel disease (IBD) is another promising indication and research in that field is still ongoing, results are so far not as promising as for CDI (22). The main difference is that it is a chronic most often longstanding disease in genetically susceptible people. A one-time FT procedure that can be effective in recurrent CDI is changing the microbiome of the recipient, but this effect does not necessarily last for a long time. In order to treat chronic genetically determined diseases, a permanent change in the microbiome would be needed (23).

FT IN CLOSTRIDIOIDES DIFFICILE INFECTION

Clostridioides difficile infections

Until 2016 *Clostridioides difficile* was known as *Clostridium difficile*. The old name is still widely used today. The renaming was done because of a taxonomic reclassification due to molecular biology related new findings after sequencing of 16s-RNA. The genus of *Clostridium* has been restricted to *Clostridium butyricum* and species related to it and therefore genera that are not within this group should be given a new name. *C. difficile* is part of the family of *Peptostreptococcaceae* and has major differences when compared to other members of *Clostridium sensu stricto*. Due to that the new genus *Clostridioides* was established and the abbreviation *C.difficile* is still in use, just its full designation now is *Clostridioides difficile* (24). *C.difficile* is an obligate anaerobic, gram positive spore forming bacterium. Apart from a mild clinical course, severe infections like pseudomembranous colitis and toxic megacolon can also be caused by the bacterium. At the end of the 1970s, *C. difficile* was identified as the causative agent of diarrhoea associated with antibiotic treatment (25). The virulence factors enterotoxin A and cytotoxin B that cause a cytotoxic damage of the intestinal cells and therefore lead to diarrhoea and colitis play a major role in disease process. The extend and severity of the symptoms also depends on several host related factors: a disturbance of gut microbiota caused e.g. by an antibiotic treatment but also other gastrointestinal diseases or interventions play a major role. Certain strains that produce a higher amount of toxins are also associated with a more severe disease manifestation. Also the immunologic status of the host and possible antibodies against enterotoxins is important in the pathogenesis (26). *Clostridioides difficile* causes approximately 15-20% of antibiotic-associated diarrhoea and over 95% of all cases of pseudomembranous colitis (27). For a definitive diagnosis, a laboratory proof is needed. The most common symptoms of an infection are a rapid onset watery diarrhoea at least three times per day for at least a period of two consecutive days with a typical putrid smell, often accompanied by abdominal pain in the lower abdominal quadrants, fever and leucocytosis and hypalbuminaemia (28). Antibiotics have been identified as one of the major risk factors for a CDI and the risk being the greatest with clindamycin followed by fluoroquinolones and cephalosporins (29). Moreover, the recurrence rate of CDI is very high and the incidence of multiple recurrent infections have been reinforcing the need and demand for further treatment options with FT being one of them (30). The *European consensus conference on faecal microbiota transplantation* from 2017 established some guidelines regarding the selection process, preparation of faecal material and administration

methods (16). The following paragraph must be seen as an overview on the general FT procedure for the treatment of especially recurrent *C. difficile* infection.

Treatment of CDI according to European and American Guidelines

First, it is important that in most guidelines FT is only recommended for a refractory episode of CDI. The *European Society of Clinical Microbiology and Infectious Diseases* recommends in their 2021 updated guidelines the use of fidaxomicin 200g two times per day for an initial episode and when not available replacement with either vancomycin 125mg four times per day or metronidazole 500mg three times per day (21). Treatment with all three regimens is 10 days and can be prolonged if the risk of recurrence is high. These recommendations are based on several trials including a Japanese study evaluated the efficacy and safety of fidaxomicin versus vancomycin treatment for CDI. In that study, regarding cure rate and risk of recurrence, fidaxomicin was slightly superior to vancomycin (31). Another trial conducted in Europe, Canada and the USA also comparing the two antibiotic agents, did not find a huge difference regarding their efficacy and only noted better results with fidaxomicin in patients receiving concomitant antibiotics for other reasons (32). For non-severe CDI the causing antibiotics agent should be stopped, and the patient should be monitored. For severe and severe-complicated CDI the *2021 guidelines* now recommend the same antibiotic scheme as for non-severe initial CDI and does not see anyone of the two to be superior to the other one. IV metronidazole addition is no longer recommended. In case oral treatment is impossible, intraluminal delivery of either vancomycin or fidaxomicin is advised. Furthermore, in patients with severe complicated CDI in whom antibiotic treatment does not show adequate results, FT should be considered. In case of recurrent CDI, the guidelines still recommend switching to another antibiotic, one that was not used in the treatment of the initial episode. In multiple recurrent CDI it is recommended to consider FT after antibiotic pre-treatment or add bezlotoxumab to the standard antibiotic treatment (21). Also, the *Clinical Guidelines of the American College of Gastroenterology* have very similar recommendations for the treatment of non-severe CDI, and vancomycin and fidaxomicin are preferred over metronidazole (33). For a severe CDI they clearly recommend vancomycin over fidaxomicin, though in the same dose as noted in the European guidelines but state that both antibiotics have yielded similar results. For the treatment of fulminant CDI, treatment with 500mg of vancomycin per oral every 6 hours (for the first 48-72 hours) is suggested and can be combined with parenteral metronidazole 500mg every 8 hours. The role of FT is explained as a separate recommendation

and should be considered in severe and fulminant CDI that is refractory to antimicrobial treatment but also for recurrent CDI in order to prevent additional relapses (33). It was proven that a pre-treatment with vancomycin 125mg 4 times daily plus FT was superior in treating recurrent CDI compared to antimicrobial therapy with either only vancomycin 125g four times per day or fidaxomicin 200mg twice per day based on their clinical or clinical and microbiological resolution (34).

FT Treatment sequence in recurrent CDI

Interestingly, as seen in Figure 1, and mentioned previously, antibiotic use is the main risk factor for a CDI, since it leads to a dysbiosis of the microbiota and a toxin mediated destruction of the epithelial cells. The main treatment method is yet again another antibiotic, which in turn makes the host more susceptible to reinfection. FT, is a treatment method that leads to an increase in microbial diversity and long-lasting effects (35) (cf. Fig. 1).

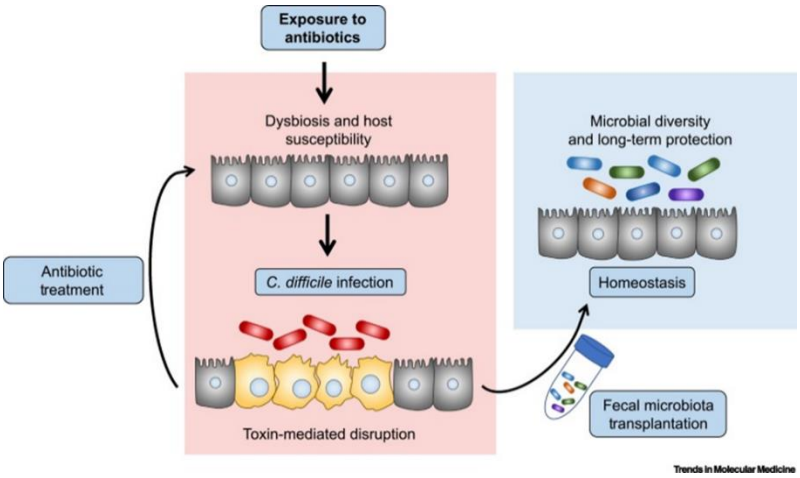

Figure 1. FT to restore microbial homeostasis (35)

A pre-treatment with the antibiotic vancomycin orally for at least 3-4 days before the FT procedure is a general recommendation in order to reduce the bacterial load of *C. difficile*. Some also suggest the use of metronidazole or fidaxomicin. There was no universal recommendation whether it should be stopped 12-48h before the stool transfer. According to *the French Group of Faecal microbiota Transplantation*, discontinuing the antibiotics before the procedure is possible but not obligatory (16,36,37). Statistically, 61% of FT centres in Europe used frozen FT preparations and only 10% of the centres opted for fresh preparations (14). This is probably related to the fact, that frozen

preparations are easier in the handling and stool banks can be established that can guarantee a more thorough screening before the transfer. The transfer material would be available when needed. Additionally, a clinical trial performed by Lee et al revealed that for the treatment of CDI the use of frozen preparations did not result in worse clinical outcomes when compared to fresh stool preparations. The randomized, controlled study enrolled 219 patients of which 108 received FT through enema with frozen faecal material and the other 111 received fresh unfrozen material. The clinical resolution was observed in 83.5% of the patients that received the frozen - and 85.1% of patients who received the fresh material coming to an end that frozen stool is not inferior to fresh stool in the FT procedure (38). Generally, the standard dose of faeces is specific to each clinic or physician. Several trials have shown that 30g of donor faeces have proven to be a sufficient amount for the FT procedure to succeed and homogenization in approximately 150ml of tab water or saline solution was recommended, in general a three to five times larger volume of solvent. In frozen preparations 150mL of saline and glycerol are added as a cryoprotective substance and stored at a temperature of -80°C for up to 16 weeks. Defrosting can be done in a 37°C water bath or at room temperature. This accounts mainly for transfer through colonoscopy (36,39). The taxonomic composition but also the viability of the microbiota remains unchanged even after storing it for six months at a temperature of -20°C to -80°C (40). Another systematic review of case series revealed that using <50g of faecal material increased the risk of recurrence by fourfold compared to procedure where ≥50g was used but very little difference in resolution rates was noted (41). After preparing the faecal material, it should be strained with e.g. a gauze to filter out any unhomogenized material (38). Another method of specimen preparation is lyophilization of the stool specimen, which is freeze-drying, to remove its water. This preparation can later be encapsulated, making a repeated administration or long-time therapy more practicable. It was proven that using a freeze-dried product without adding a cryoprotectant in the process reduces the efficacy of FT when compared with using frozen product or fresh stool (42). The *French Group of Faecal microbiota Transplantation* established a treatment sequence and also recommends performing a bowel cleansing with 4L of a solution containing a laxative for delivery by colonoscopy (37) (cf. Fig. 2).

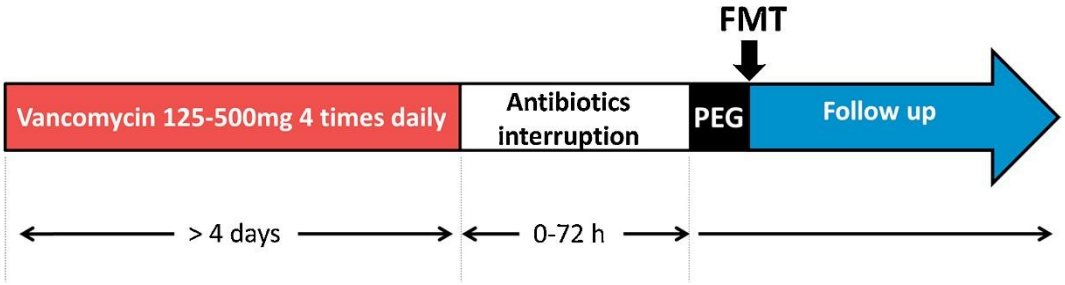

Figure 1. FT treatment sequence: Pretreatment with Antibiotic. PEG: polyethylene glycol. FT procedure (termed FMT in the picture) (37)

Methods of administration

The main methods of administration used in the treatment of recurrent CDI by FT are colonoscopy, enema, nasogastric tube and the only non-invasive method - oral capsules. A randomized clinical trial by Cammarota et al. reported after administration of FT via coloscopy a cure rate of 65% after the first FT procedure and 90% after multiple procedures. It was reported that two people participating in that study died from *C. difficile* associated complications (43).Another randomized clinical trial by Kao et al. compared Oral Capsule and Colonoscopy delivered FT. 105 adults with recurrent CDI that were randomly assigned one of the two methods of delivery completed the trial. In both groups a cure rate of 92.2% was seen, suggestion non-inferiority of either of the two methods but more patients in the "oral capsule group" rated their treatment experience as "not unpleasant". Due to the treatment success, only one procedure was performed (44). Also Hirsch et al. proved that orally administered capsules are very effective in the treatment of recurrent CDI (45). Lee et al. evaluated the efficacy of FT via enema and the cure rate after the first procedure was only 47.9% while the cure rate after multiple procedures reached 86.2% (46). Further results of trials can be seen in the table below (13,47–49). The clinical trial is indicated in the first column, followed by the success rate after one FT procedure, the success rate after multiple procedures and the fourth column was filled if the study results only specify the overall treatment results. In most trials a cure rate is understood as the resolution of CDI associated diarrhoea and the prevention of its relapse (cf. Table 1).

Table 1. Comparison of methods of application of FT

Clinical Trial	**Method of administration**	**Cure rate after first FT procedure in %**	**Cure rate after multiple FT procedures in %**	**No information about the number of procedures: success rate in %**
Cammarota et al. 2015	Colonoscopy	65%	90%	
Kao et al. 2017	Colonoscopy	92.2%	-	
Youngster et al. 2014	Colonoscopy	80%	100%	
Nood et al. 2013	Colonoscopy	81%	94%	
Jiang et al. 2018	Oral Capsules	63%	91%	
Kao et al. 2017	Oral Capsules	92.2%	-	
Hirsch et al. 2015	Oral Capsules	68%	89%	
Lee et al.	Enema	47.9%	86.2%	
Jiang et al. 2018	Enema	-	-	88%
Youngster et al. 2014	Nasogastric Tube	60%	80%	
Kronman et al. 2015	Nasogastric Tube	-	-	90%

It is evident in table 1 that the success rate of FT via Colonoscopy varies from 90% to 100% making it the most effective method of application when compared to the others regarding the evaluated clinical trials. Regarding the oral capsules, the cure rate was 89-91% and for the enema 86.2-88% and application of FT via nasogastric tube resulted in resolution of symptoms in 80-90% of the

patients. It is clearly seen that FT achieved results above at least 80% in treatment of recurrent CDI, and all methods are therefore deemed to be an effective treatment method. Evidently, some patients need to undergo repeated FT. Without a doubt, the method of administration via oral capsules, the only noninvasive method of FT transfer, would be a method to relieve healthcare workers, avoid the hassle of finding and screening possible donors and is probably also less stigmatized, since most patients are used to taking oral tablets in their everyday life as a method to treat a disease or its symptoms. Capsules therefore combine the advantages of both treatment methods for CDI – the convenience of using tablets and the cost-effectiveness and efficacy of using FT just with a different method of application. Varga et al. in their clinical trial compared different FT methods in order to find out how to apply it more effectively, conveniently and just have more flexibility (50). For a long time, invasive methods like colonoscopy have been the only way of administering FT but this trial found out that capsules containing lyophilized supernatant are non-inferior to other modalities of transferring the fecal microbiota. Furthermore, the capsules can be stored for up to one year at a temperature of -20°C which can ensure a continuous stock and immediate use of the medication. Nevertheless, the choice of the delivery modality must be made on accordance with the preferred method of the patient, the circumstances and most importantly the opportunities of the healthcare provider.

Comparison of Antibiotic Therapy and FT

Especially since Nood et al. published a randomized controlled trial in 2013 in patients with recurrent CDI, FT has been established as a good new treatment method. FT via nasoduodenal probe was proven to be superior over conventional therapy with vancomycin when including 43 patients regarding their therapeutic response and rate of recurrence (81% sustained response in FT vs 31% in vancomycin) (13). Cammarota et al. compared FT by colonoscopy with vancomycin for the treatment of recurrent CDI. In the vancomycin arm, only 26% of patients exhibited a resolution of CDI whereas in the FT by colonoscopy arm, 90% exhibited that resolution (43). In addition to the high effectiveness of FT in recurrent CDI, there is also a cost advantage when compared to antibiotic therapy with Vancomycin or Fidaxomicin. In their cost-effectiveness analysis, Lapointe-Shaw et al. compared six treatment strategies for recurrent CDI: the three oral antibiotics metronidazole, vancomycin, fidaxomicin and FT by either enema, nasogastric tube, or colonoscopy. The results show that FT by colonoscopy was less costly and overall more efficient than all other treatment methods compared, followed by FT by enema (51). Another Australian

study stated that nasoduodenal and colorectal FT improved the quality of life more while also being at a reduced cost compared to antibiotic treatment with vancomycin (52).

Side effects of FT in recurrent CDI

In general, FT is a rather safe procedure and unwanted adverse effects are rarely occurring. In 2019, the *FDA* reported about the transmission of multi-resistant bacteria, namely extended-spectrum beta-lactamase producing *Escherichia coli* via FT in two patients who had undergone two different clinical trials. Consequently, one of the patients died. It was later found out that both stool recipients received stool of the same donor who had never been tested for multi-resistant germs. This incident reinforces the need for adequate donor screening (53). Some adverse events after FT reported are constipation, mild abdominal pain, bloating, transient mild diarrhea, nausea and vomiting. In one trial, two urinary tract infections were reported (13,43,47). A case series of FT in immunocompromised patients with CDI reported no serious adverse events. Mild abdominal pain and a disease flareup was reported in some patients. As a matter of course, the methods of application of FT themselves pose a risk to the patient. Side effects of sedation for invasive stool transfer and colon perforations and bleeding during colonoscopy have been occasionally described (54). In one patient, fatal aspiration pneumonia was the consequence of FT administration via nasoenteric tube and following regurgitation of the fecal material (55). The *American College of Gastroenterology* therefore does not recommend FT via nasoenteric tube (33).

OTHER FIELDS OF APPLICATION IN CLINICAL MEDICINE

Inflammatory Bowel disease

Inflammatory bowel disease is a chronic disease of which the two main entities are Ulcerative Colitis and Crohn's disease. They are characterized by chronic reoccurring inflammation of the Gastrointestinal tract but are not only limited to it as they are considered an inflammatory multisystem disease. IBDs are complex diseases, and their genetic factors and environmental factors play a role in the pathogenesis. The mucosal immunologic system of the gut is activated in genetically susceptible individuals that are triggered by environmental factors, a clear pathophysiologic mechanism though is still obscure (56). Also the gut microbiome is involved in the pathophysiology – it is suggested that in patients with IBD the present imbalance of the gut microbiota, the so-called dysbiosis plays a major role (57). Even though IBD could not be attributed

to any of the traditional pathogens, they certainly are involved in the pathogenesis of IBD. Treating that dysbiosis by manipulating the intestinal microbiota with FT therefore could be a possible approach in the treatment of IBDs.

Ulcerative Colitis

Several randomized controlled trials with FT for the treatment of UC have been performed. The clinical trial by Moayyedi et al. from 2015 randomly assigned 70 patients with active UC to two different options: FT via enema or a placebo enema with water. This procedure was carried out once a week for a period of six weeks. Remission of UC was achieved in 24% of the patients that received FT and in 5% of the patients receiving the placebo. Furthermore, after completing the trial the microbial diversity of the patients that had received the actual stool transplant was greater (58). Another randomized clinical trial by Costello et al. completed by 69 adults showed similar results. The two treatment arms were either FT from a pooled donor or autologous FT via colonoscopy which the patients were assigned to randomly. Among the patients receiving the donor transfer, 32% saw a disease remission whereas only 9% from the autologous group saw the same effect (23). The methods of application in the two previous studies, enema, and colonoscopy, were both invasive and required the patient to go see a health specialist every time. The rather new method of administering FT by oral capsules presents a promising approach in the treatment of UC and could ensure a long-term, convenient and patient oriented therapy. A randomized pilot study from 2021 compared two treatment arms: six patients received FT by colonoscopy as induction therapy followed by a maintenance therapy of daily oral frozen FT via capsules for 12 weeks and another six patients a placebo. As a result, two out of the six patients receiving the active treatment have achieved a remission in UC and a sustained change in their faecal microbiota composition versus none in the placebo group (59). These outcomes indicate that administering daily oral FT may indeed lead to a more permanent change in the gut microbiota and is a promising treatment approach. Larger trials should be conducted.

Crohn's Disease

Also, in the pathogenesis of CD, the gastrointestinal microbiota is increasingly recognized to play a principal role, but little research has been conducted regarding FT as a possible approach to achieve and maintain remission. A pilot randomized controlled study from 2020 included two adult patient groups, of whom eight patients received FT by colonoscopy and nine a placebo. The

primary endpoint of the trial was the implantation of the donor microbiota at week 6, but none of the patients have reached it. Less patients in the FT group experienced a flare of their disease than in the sham group and the severity of the disease decreased more (60). Yang et al. compared FT via gastroscopy with administration via colonoscopy as a potential therapy for CD. Out of all patients included in the trial, remission of the disease was achieved in 66.7% of all patients and no noteworthy difference between the two methods of administration was observed. The patients expressed a greater microbial diversity after the transplant but compared with the healthy donors expressed lower levels of certain bacteria. No dangerous adverse effects were noted (61).

Psychiatric diseases

The human gut has many different functions and over the last decade the intestinal microbiota has been recognized as one of the key regulators of the gut-brain axis. This axis is a rather new concept in research and describes a bidirectional communication between the gut microbiome and the brain. This compound concept is not yet fully understood but is thought to involve several players like the gastrointestinal system with its microbiome, the central, autonomic and also enteric nervous systems, the immune and the neuroendocrine system (62). The knowledge regarding this concept has broadened and the term microbiota-gut-axis is being used more and more. The human gastrointestinal tract and especially the gut are heavily populated by trillions of intestinal microbes, mainly bacteria (2). The GI microbiota may influence the human behavior and may play a role in the pathophysiology of psychologic disorders. To establish causality, several animal trials have been conducted. One example would be the induction of gut dysbiosis by administration of an antibiotic in adolescent mice. The resulting depletion of gut microbiota led to a reduced anxiety but emerging cognitive deficits and also altered several neuromodulators important in the gut-brain axis communication. Brain development in these mice therefore was abnormal due to the lack of gut microorganisms (63). Another trial that tested the correlation between gut microbiota and depression involved depressed and healthy patients. Stool samples were collected from both groups and later transferred to a rat model. The stool of the depressed patients was less microbiota rich and when transferred to the rodents, induced depression-like symptoms in these animals (64). Due to promising correlations between microbiota and psychiatric effects in animals, microbiota-oriented treatments could be a promising track in the treatment of mental disorders and therefore FT may be useful in the treatment and counteraction of psychiatric illnesses.

Autism spectrum disorder is a disorder that is characterized by modified social communication and interaction and repetitive stereotyped behavior (65). Several studies have shown that autism symptoms also reflect in the composition of the patient's microbiome, therefore making it promising new therapeutic approach in the treatment of the pathology (66,67). Furthermore, children with ASD often exhibit a disturbance of their gastrointestinal system and more frequently display symptoms such as abdominal pain, gaseousness, pain on stooling, constipation and diarrhea when compared to typically developing children (68). Several recently published studies have proven an improvement of not only gastrointestinal symptoms but also autism symptoms following an FT procedure. Li et al. have shown in their open-label clinical trial that involved 40 children with ASD, who also displayed GI symptoms, and also an age-matched typically developing control group. The duration of the study was 12 weeks in total including a 4-week FT treatment phase followed by an 8-week observation phase post-FT. The two methods of administration were freeze-dried capsules and for those children who were unable to swallow capsules, colonoscopy. After the FT treatment, the Gastrointestinal Symptom rating scale, the Bristol stool scale and the Daily Stool Record were used to evaluate GI symptom improvement, and it showed that The GSRS scores had decreased by 35% and also the stool properties had improved significantly. Autism spectrum symptoms improvement was evaluated with the Autistic Behavior Checklist and Childhood Autism Rating Scale and both showed an improvement regarding the score. The effects of both Gastrointestinal and Autism symptoms improvement were stable also during the 8 week follow up phase. Merely the Social Responsiveness Scale improvement results were reversed without further FT treatment. FT was well tolerated among the children, no severe complications were noted. Interestingly, also a change in neurotransmitters was noted. 5-HT and GABA concentrations in serum had decreased post FT while DA levels had increased. This is a prove that the gut and brain are indeed connected through the microbiota-gut-axis (69).

Regarding Major Depressive Disorder (MDD), large-scale trials with FT as a possible treatment option are still missing but several case reports can be found. A 79-year-old woman with mental depression received a stool transplant via gastroscopy from a healthy donor. After the FT procedure, the patient's depressive symptoms had improved and RNA sequencing showed a change in the gut microbiota composition of that patient (70). These results must be proven in a larger scale trial.

Metabolic syndrome

Obesity especially in western countries has become a major public health issue. Interestingly, alterations of the intestinal microbiota are also associated with obesity and insulin resistance, and it was found that altering the caloric intake of humans, causes dynamic changes of gut microbiota (71). Lean individuals who increased their caloric intake had an increase in *Firmicutes* and decrease in *Bacteroidetes*. Evidently, the nutrient load thus is influenced by the nutritional load. A Dutch based study investigated the correlation of the microbiome and insulin resistance. In that large scale cross-sectional study including 2166 participants, a higher microbiome diversity together with an increased number of butyrate-producing bacteria in the gut was connected with a lower incidence of insulin resistance and type 2 diabetes mellitus (72). Another Dutch trial published in the year 2012 investigated the effects of transplanting stool from healthy, slim individuals to recipients with metabolic syndrome by observing the changes in microbiota composition and glucose metabolism. The participants were randomized and assigned either allogenous or autologous FT. Six weeks after having been transplanted the stool of healthy non-diabetic individuals, the insulin sensitivity of the recipients with metabolic syndrome has improved a lot and the percentage of butyrate-producing bacteria increased (73). From a metabolic standpoint, it is thought that the gut microbiota influence the lipid accumulation, the lipopolysaccharide content and the production of short chain fatty acids like butyrate, that in turn influence inflammatory reactions or the insulin metabolism (74,75). On the contrary, another trial where FT was administered via capsules from lean donors or placebo to adults with obesity, found no significant discrepancy between the two groups regarding their glycemic outcomes, weight or body composition over a 12-week period. Merely a minor improvement in HbA1c in the FT treatment arm compared to the placebo was reported (76). The disappointing result might be since the method of administration of FT in this trial was oral capsules. Additionally, differences between the study populations or the engrafting microbiota might explain the overall result. Nevertheless, these findings might provide a new therapeutic approach, where FT could potentially have an important place but ongoing research trials to further prove or refute a beneficial effect on FT in metabolic syndrome need to be conducted.

Cancer

Over the past decade, a lot of new scientific knowledge has been acquired regarding the etiology of cancer and the intestinal microbiome has been attributed some influence in its pathogenesis.

Especially the interactions of neoplastic cells, immune cells and bacteria and the effects of diet and antibiotics on the carcinogenesis are a hot topic in research (77,78). Since it is known that FT has the potential to counteract microbial dysbiosis in the gut and does work well in the treatment of refractory CDI, there is potential that cancer patients might also benefit from the procedure. A recent Israeli based study by Baruch et al. investigated the effect of FT in immunotherapy non-responders. Patients with advanced metastatic melanoma who did not respond to the treatment with anti-programmed cell death protein 1, an immune checkpoint inhibitor and a type of immunotherapy, received a fecal transplant from a patient who did respond to the treatment. As a consequence, 6 out of the 15 participants in the trial had a clinical benefit from the procedure and exhibited an increased amount of intestinal bacteria that are shown to be affiliated with a better response to that kind of immunotherapy (79). Further research needs to be conducted to understand what kind of components of the microbiome are responsible for the therapeutic effect. Chemotherapy in cancer patients is linked with a lot of different side effects with especially gastrointestinal toxicity being extremely harsh and bothersome. Nausea, vomiting, diarrhea, constipation and mucositis are some of the most commonly reported side effects (80) and not only affect the quality of life during chemotherapy of the patient but can also delay or postpone the different treatment cycles. A disruption of the gut microbiota due to cancer therapy and other drugs would be a feasible indication for FT as a supportive treatment in oncology, especially since it has proven to be safe also in immunosuppressed cancer patients (81). The only study to this date to investigate FT as a possible treatment for GI associated side effects in chemotherapy was conducted by Bastard et al. and involved mice that were exposed to the chemotherapeutic agents 5-fluorouracil and antibiotics. Following the microbiome transplant, a notable increase in bacterial species that show anti-inflammatory effects was noted but no clinical endpoints like e.g. a reduction of diarrhea were documented (82). The results show that FT could be a promising novel treatment to alleviate GI-toxicity in cancer patients.

MECHANISM OF ACTION OF FT

Fresh faeces have a water content of approximately 75% and out of the remaining 25% around 84-93% are organic solids. These solids are comprised of 25-54% bacterial mass, 2-25% protein or nitrogenous substance, 25% of carbohydrates or fibre and 2-15% of fat. Furthermore, epithelial cells, mucus, calcium and iron phosphates also make up a small part of faecal matter (83). Notably, the bacterial mass makes up a rather large percentage of the overall faecal matter and has been

thought to mainly be responsible for the success of FT. Currently, that procedure is only routinely used in the treatment of recurrent *Clostridioides difficile* infection. To apply it to other diseases beyond CDI, its mechanism of action has to be understood. The diseases that can or could potentialy be treated with FT all have one thing in common: they are associated with a disbalance of faecal microbiota. The main idea of FT is that the donor stool microbiota engrafts into the recipient's gut and displaces the disease-causing organisms or simply leads to a greater species richness in the gut and therefore cures the disease and the with it associated disbalance (84). In refractory CDI, the dysbiosis is strongly pronounced, hence healthy donor stool can displace the pathogenic disease-causing organisms. Before the transplant and while still being infected with *C. difficile*, the dominant microbiota detected were *Proteobacteria* and *Bacilli*. After FT it was dominated by *Bacteroides* and *Clostridium* groups and also butyrate-producing bacteria (85). Butyrate, a short chain fatty acid, has multiple beneficial effects on especially the gut: it regulates the transepithelial transport of liquids, mitigates mucosal inflammation, strengthens the epithelial defence barrier and also has a positive impact on the intestinal motility (74). The increase and change in microbial diversity and rather fast normalization of symptoms post FT in refractory CDI, is a proof that the donor microbiota becomes engrafted into the recipient's gut. One hypothesis of how FT works in the treatment of refractory CDI, is niche exclusion. The microorganisms of the healthy donor faeces compete for nutrients with the disease causing organisms and research is hoping to use isolated non-toxigenic *C. difficile* stains to cure CDI and displace the disease causing toxigenic *C. difficile* (86). It is also proven than FT results in reinstatement of secondary bile acid metabolism, where components that inhibit *C. difficile* spore germination and expansion are created. Interestingly, bile acids are produced by the liver and metabolized by the microbiota. Whereas the already metabolized secondary bile acids inhibit the germination of *C. difficile* spores and therefore act as a protective factor against a CDI, the not-yet metabolized primary bile acids induce that very same spore germination. In patients with CDI, high levels of primary bile acids and a deficiency of secondary bile acids were detected and that imbalance, among other things, can be restored with the FT procedure (87). Moreover, also different immune-mediated pathways are thought to be involved in the restoration of gut homeostasis. A trial with mice concluded that FT induces the expression of IL-25 in the colon and post-FT the microbial diversity and the levels of IL-25 were increased, and additionally homeostatic genes were expressed while inflammatory genes were repressed (88). Also in IBD significant changes in microbiome, most likely a reduced species richness, can be observed in affected patients, and new sequencing methods for a more

detailed description of the dysbiosis have improved the overall understanding of the importance of the intestinal microbiota in disease and health (89). Notably, FT as a treatment method of IBD has yielded less promising results when compared to recurrent CDI treatment (23,58–61) suggesting that there must be other etiological factors that play a role in IBD apart from bacterial dysbiosis or that bacterial engraftment simply did not happen in these patients. A recent German based case series used sterile-filtered stool as a transplant, where small particles and bacteria had previously been removed to treat patients with symptomatic chronic-relapsing CDI. The transplant in that case is called faecal filtrate transfer. All five patients enrolled in the trial had a normalization of their stool frequency and a reduction of their symptoms following the bacteria-free stool transplant (90). From this trial one can deduct that also other components of human stool like bacterial components, metabolites and also bacteriophages play a key role in the pathophysiology of stool transplants and maybe faecal filtrate transfer can be used especially in immunocompromised patients, in whom exposure to some bacterial stains might be detrimental. This raises the question if research should not strictly focus on bacteria as their main interest but also on members of non-bacterial domains of life and once again emphasizes that fecal transplantation is indeed a more suitable term than fecal microbial transplantation for the procedure. Bacteriophages are another numerically dominant and diverse member of the faecal microbiota. Few trials have investigated the extent to which these viruses colonise the recipient's gut after an FT procedure, and most research has been focusing on the bacterial colonization. A study where FT was conducted to successfully cure CDI analysed the patient and donor stool samples regarding their bacterial and bacteriophage composition and concluded that bacteriophage abundance at least partially influences the success of FT (91). There are several other theories about the mechanism of action of FT, and research so far has mainly focused on understanding how FT works in the case of CDI. The exact pathophysiologic mechanism is expected to go a lot deeper than expected and ongoing research is being conducted to have a better understanding and maybe also use the procedure in the treatment of other diseases.

CONCLUSION

While fecal transplantation has become crucial in the treatment of refractory and recurrent *Clostridioides difficile* infection, other indications for the procedure are still missing. Although it has been proven that the gut microbiome is indeed dysbalanced in many pathologies, no clinical trial that investigated the therapeutic effect of fecal transplantation in other diseases has yielded as

promising results as in the treatment of *Clostridioides difficile* infection. When compared with standard antibiotic treatment for the disease, fecal transplantation yielded better results. It is important to mention, that also the exact pathophysiologic mechanism for diseases like inflammatory bowel disease has not yet been fully understood and the gut disbalance might only be a consequence of another, more complex etiology. In that case the treatment with fecal transplantation might only be a symptomatic one and not target the origin of the problem. Furthermore, regarding psychological diseases, the concept of the gut-brain or respectively the microbiota-gut axis is also a rather new one and further research needs to be conducted. Promising results were achieved with fecal transplantation in patients with autism spectrum disorder, who not only showed an improvement regarding their gastrointestinal but also their autism symptoms. An auspicious concept, yet without any human trials to this point, would be the use of fecal transplantation to alleviate the gastrointestinal symptoms in patients undergoing chemotherapy, since it has proven to be safe also in immunocompromised patients or a sterile-filtered stool could be used.

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