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REVIEW

FECAL MICROBIOTA TRANSPLANTATION IN ADULTS AS A MODERN FORM OF PAST "COPROTHERAPY": HOPE OR HYPE?

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Abstract: The influence of intestinal microbiota on human health and disease is of great importance. Fecal microbiota transplantation (FMT) defined as the transfer of the stool-derived microbiota of the distal gastrointestinal (GI) tract from a healthy donor to a patient with a disease attributable to intestinal dysbiosis is, in addition to the use of probiotics, prebiotics, synbiotics and eubiotics, one of the methods to restore eubiosis. Thorough medical history and physical examination followed by a set of blood and stool laboratory tests should be performed in a potential stool donor. Stool-derived microbiota may be administered through the upper and/or lower GI tract. FMT is believed to be a well-tolerated and, in general, safe procedure. The emergence of stool banks of frozen feces-derived material containing intestinal microbiota and the availability of convenient oral capsules with selected components of feces would definitely facilitate the use of this method in both research and the clinics. An inflammation caused by *Clostridium difficile* is the most often indication for FMT. Other conditions include inflammatory bowel disease, irritable bowel syndrome or the eradication of multi-drug resistant microorganisms. However, the list of potential indications rapidly increases. Further randomized double-blind studies in humans are needed to confirm a real benefit-risk ratio and clinical value of FMT, especially in extraintestinal disorders like obesity, diabetes mellitus, metabolic syndrome, fatty liver disease, hepatic encephalopathy, allergy, autism, depression or dementia.

Keywords: intestinal microbiota, dysbiosis, fecal microbiota transplantation, *Clostridium difficile*, inflammatory bowel disorders, irritable bowel syndrome

The intestinal microbiota consists of all microorganisms that inhabit the gut: bacteria, viruses, fungi, and protozoa (1). This complex ecosystem is represented primarily by approximately 1000 species of bacteria, mainly *Bacteroidetes*, *Firmicutes*, *Proteobacteria,* and *Acinetobacter* (2). The number of bacterial cells in the large intestine exceeds ten times the number of human cells (3). It is believed that the right qualitative and quantitative composition of the intestinal microbiota and the correct interactions between the macroorganism and the GI microorganisms are absolutely necessary to maintain good health. There is growing scientific evidence that not only bacteria but also fungi (gut mycobiota) and viruses, primarily bacteriophages, constitute an important part of microbiota in health and disease. It has been found that yeast *Candida albicans* may stimulate *Bacteroides spp*. growth and change its sensitivity to chloramphenicol whereas probiotic yeast *Saccharomyces boulardi* promotes a healthy shift of gut microbes in different clinical conditions (4). Studies on still poorly understood bacteriophages-bacteria-host interactions disclosed that even viruses that infect prokaryotic cells alter gut microbiota to such an extent that they may play a role in the etiopathogenesis of numerous disorders including inflammatory bowel disease (IBD) (5).

At present, the etiopathogenesis of many gastrointestinal (GI) and non-GI diseases is associated with dysbiosis, i.e. impaired composition and function of the intestinal microbiota. Although the characteristic individual pattern of microbiota alterations for specific disease entities has not been established, the reduction of microbial biodiversity is the most constant feature of concomitant dysbiosis present in many pathological conditions. Recognition of the role of microbiota in health and disease prompts methods of its beneficial modifications, including not only the use of probiotics, prebiotics, synbiotics,

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and eubiotics, but also fecal microbiota transplantation (FMT), the latter described in the review.

Intestinal microbiota, which has evolved with humans for thousands of years, is its integral part that plays an important role in physiology, including metabolism, immune function, and maintenance of intestinal homeostasis. The beneficial effects of a diverse but balanced microbiota include the development of the newborn and infant's immune system, food tolerance, prevention of autoimmune diseases, stimulation of GI maturation and intestinal barrier, prevention of infection, development, and maintenance of normal GI motility and visceral sensation, improvement of the absorption of food ingredients or production of certain vitamins and short-chain fatty acids like butyric and propionic acid.

The relatively constant and highly individual qualitative and quantitative composition of the microbiota is subject to constant, but slight fluctuations under physiological conditions. Several external factors including drugs may alter normal human microbiota. Recent studies show that some antibiotics have a long-term impact on intestinal microbiota assessed in stool samples. Jakobsson et al. found that a one-week treatment with clarithromycin and metronidazole results in a radical decline in *Acinetobacter spp*. population in feces and promotes post-treatment antibiotic resistance to macrolides detected even four years later (6). Scientific observations from all over the world confirm frequent misuse and overuse of proton pump inhibitors (PPIs), also available as over-the-counter preparations (7). Gastric acid suppression increases the risk of gut microbiota disequilibrium and enteric infections. *Firmicutes*, especially *Streptococcus spp*., are more abundant in gastric microbiota compared to the control group not exposed to PPIs (8). Moreover, PPIs users are at increased risk of small intestine bacterial overgrowth (SIBO), *Clostridium difficile* infection (CDI), candidiasis, traveler's diarrhea and, in case of coexisting liver cirrhosis, spontaneous bacterial peritonitis. Thus, any significant, unfavorable quantitative and qualitative changes of microorganisms constituting the complex intestinal ecosystem, referred to as dysbiosis, especially reduction of their natural biodiversity, contribute to the development of many diseases, both extra- and gastrointestinal. It is believed that dysbiosis may play a role in the etiopathogenesis of metabolic syndrome and its compounds like diabetes, obesity or nonalcoholic fatty liver disease. High prevalence of intestinal dysbiosis resulting in SIBO, increased endogenous ethanol production and intestinal permeability, bacterial translocation, higher blood concentration of proinflammatory bacteria-derived products like lipopolysaccharide have been described in chronic liver damage (9). Gut dysbiosis was reported as a putative factor for the development of depression, neurodegenerative diseases, autism, or schizophrenia, although it is worth noting that dysbiosis even in digestive tract diseases is rarely the only etiological factor (10).

Dysbiosis-induced disorders may be prevented or treated by restoring the physiological state of the intestinal microbiota, i.e. eubiosis. Methods for the beneficial microbiotic modification include the use of probiotics, prebiotics and synbiotics, the intake of specific antibiotics that are not absorbed from the GI tract, such as neomycin and rifaximin, and the transplantation of a healthy human intestinal microbiota known as FMT (11).

FMT is defined as the administration of fecal material containing microbiota of the distal GI tract (large intestine) from a healthy person (a donor) to a patient with a disease or disorder associated with intestinal dysbiosis (12). The goal of FMT is to treat the disease by restoring the biodiversity and microbiota typical of a healthy person. It has been proven that this transfer improves the composition of the intestinal microbiota (13, 14).

Interestingly, the concept of FMT is not entirely modern. First descriptions of FMT come from China and date back to the 4th century AD (15). At that time, this method was used to treat food poisoning and severe diarrhea. In the 17th century, FMT was used in veterinary medicine (16), whereas in 1958 four children suffering from severe pseudomembranaceous colitis (PMC) were treated (17). In 1983, the case of successful treatment of CDI with FMT was described (18). Data on the use of FMT as a rescue method in CDI were initially casuistic, but its effectiveness, as well as the growing evidence on the role of dysbiosis in the pathogenesis of many gastroenterological and non-GI diseases, increased the interest in FMT of researchers, clinical practitioners, patients and pharmaceutical industry (19, 20).

It is important to stress that according to recently published international guidelines on stool banking for FMT, stool banks should appoint a pharmacist (coordinating the management of stool samples from the processing of feces in the laboratory up to storage) as members of the scientific committee (21).

FMT procedure

It is worth emphasizing that FMT procedure should follow specific high-quality standards (22). Although preferences as to the choice of a stool donor, the list of tests performed before donation and technical details may differ between medical centers, it is strongly recommended to adopt current national or international consensus guidelines on FMT in clinical practice (21, 23).

Donor selection

The selection of a proper donor is the key to successful FMT. Both, an adult related to the host or a stranger may serve as the donor of intestinal microbiota. The choice of a donor (a spouse/relative or a stranger) usually depends on practical considerations, but from a medical point of view donation from a particular person has advantages and disadvantages. If the fecal donor is a spouse of the ill, the donor and the host are generally exposed to the same environmental risk factors, which can reduce the risk of pathogen transmission. In turn, a 1st degree relative in the maternal line has the most components of intestinal microbiota shared with the host. A male donor is potentially better, because, at least in theory, the components of the female intestinal microbiota may play a role in the pathogenesis of autoimmune diseases and IBS (higher incidence in women compared to men, especially with regard to systemic connective tissue diseases). There is no need to select the stool donor in terms of gender and age, but to diminish the risk of comorbidities potential donors aged < 60 years should be preferred (23). The choice of a healthy, but unrelated donor may be more beneficial in those entities in which genetic factors play a role in the etiopathogenesis, e.g. in IBD, especially in Crohn's disease. On the other hand, family members of the patient or his/her spouse may conceal risky sexual behavior or other medical information, thus creating a higher risk of infection of the host transferred by biological material.

Pre-donation studies

The stool donor should be thoroughly examined. Potential donors should complete a written questionnaire containing a set of questions focused on their medical history and risky lifestyle habits to identify exclusion criteria following the requirements of the European Commission for the selection of human tissue transplants like, for example: a) drugs that may impair gut microbiota – chronic ≥ 3 months) daily therapy with PPIs, recent ≤ 3 months) exposure to antibiotics, chemotherapy and immunosuppressants; b) GI, metabolic and neurological disorders and c) infectious diseases (21, 22, 23). Previously screened donors should undergo a thorough interview again on the donation day in order to address a recent onset of alarming signs, symptoms, or risky behavior.

Table 1. Selected exclusion criteria and recommended blood and stool testing in nonspecific situations for potential stool donors (23).

Exclusion criteria	Donor test
Recent (= 3 months) antibiotic therapy Gastroenterological diseases, including: IBD, IBS, chronic constipation Major GI surgery Autoimmune disease Atopy/allergy or immunomodulating treatment History of chronic pain syndromes (fibromyalgia, chronic fatigue syndrome) Neurological diseases and neurodevelopmental disorders Metabolic syndrome, obesity, moderate and severe malnutrition Malignant neoplasms in the past or during treatment, regardless of location	<u>Blood tests:</u> – HAV, HBV, HCV, HEV – CMV, EBV – HIV-1, HIV-2 – syphilis – complete blood count – CRP, erythrocyte sedimentation rate – albumin, creatinine and electrolytes – aminotransferases, gamma-glutamyltransferase, alkaline phosphatase, bilirubin <u>Stool tests</u>: – *Clostridium difficile* – enteric pathogens including *Salmonella* i *Shigella, Campylobacter, E. coli O157 H7, Yersinia,* vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus,* Gram-negative multi-drug resistant bacteria – Norovirus – *Giardia lamblia* and *Criptosporidium parvum* – helminths and protozoa – fecal occult blood test

A stool donor should be free from diseases that, even theoretically, may be transmitted with feces. A number of infectious diseases must be excluded with blood and stool tests performed at most 4 weeks preceding donation (Table 1).

In certain situations, depending on both the medical history of the potential donor and the clinical condition of the host, e.g. severe immunosuppression, the list of tested pathogens may be expanded, for example, *Isospora, Microsporidia, Strongyloides stercoralis, Listeria monocytogenes*, *Vibrio cholerae*, *Helicobacter pylori*, rotavirus, HTLV type I and II or calprotectin (23).

Stool preparation

A minimum amount of 30 g of feces should be used. Fresh stool that requires dilution, homogenization and filtration (at ambient temperature of 20-30 centigrade degrees and in anaerobic conditions whenever possible) should be used within 6 h after defecation. Saline, the least harmful to intestinal microbiota, is the most preferred solvent. Protection (gloves and facial masks) should be used during preparation. The fecal suspension is either directly administered into the digestive tract of the host or subsequently processed and stored at -80 centigrade degrees with glycerol as microbial cryopreservant, ideally in stool banks that fulfill high standards according to the international consensus (23). In the latter case, fecal suspension should be thawed in a water bath at 37 centigrade degrees and infused within 6 h (21). Preclinical study reports declined microbial viability in frozen feces after 9 months (24). Therefore it is suggested to use frozen stool samples within 1 year from the donation (23).

Transfer of the human-derived intestinal microbiota

The intestinal microbiota is administered through the upper GI tract – via gastroscope, nasogastric, nasojejunal or gastrostomy tube or orally as capsules, if available, or through the lower GI tract – to the proximal part of the large intestine via colonoscope, to the distal part of the large intestine via enema or both (upper and lower GI) routes. Bowel lavage with polyethylene glycol should precede FMT.

Administration with a nasogastric or nasojejunal tube may be uncomfortable or repelling, requires radioscopy to confirm the position of the tube, and involves the risk of complications associated with the procedure, e.g. vomiting and aspiration of contents into the respiratory tract. Intestinal obstruction excludes the administration of biological material through the upper GI tract. The advantages of rectal infusion are low costs, low risk of procedure-induced complications, but some patients may find it difficult to maintain the fecal suspension and need repeated infusions.

The endoscopic fecal transfer is well-tolerated and additionally allows assessment of the GI mucosa. However, the disadvantages include the risk of complications depending on the endoscopic procedure itself and increased FMT costs (22). Both methods are effective, although less invasive approaches (oral or rectal) may be easier to perform or more acceptable by some patients.

The relative simplicity of the endoscopic-independent way of fecal transfer means that, theoretically, FMT can also be performed in outpatient clinics. Moreover, FMT is performed at home as a do-it-yourself procedure, which, of course, is not recommended, but, as instructional videos on the internet show, it happens in real life.

Indications for FMT

Clostridium difficile infection

Due to the limited selectivity of antibacterial drugs, not only the number of pathogens but also beneficial bacteria in the digestive tract are affected by antibiotics. Antibiotic-induced intestinal dysbiosis may lead to antibiotic-associated diarrhea (AAD) defined as diarrheal syndrome occurring during or up to two months after the withdrawal of antibiotics. This occurs in approximately 30% of patients (25, 26). The selection of resistant bacterial strains, most often *Clostridium difficile* (15-25%) results in *Clostridium difficile*-associated diarrhea (CDAD), including PMC found in 1-5% of patients with AAD. The incidence of PMC in developed countries is increasing (27, 28). The risk factors for AAD and CDAD include the use of broad-spectrum antibiotics (especially some cephalosporins, fluoroquinolones and clindamycin), long-term antibiotic therapy (especially over 4 weeks), the coexistence of numerous diseases, and acquired or congenital immunodeficiency.

Certain forms of CDI: with repeated relapses, no response to standard therapy or severe manifestation not responding to intensive treatment within 24-48 h are currently the best documented indication for FMT (12, 23). FMT is also an effective method of preventing CDI relapses. Numerous case reports and case series, followed by randomized trials and their meta-analyses, show that in severe or recurrent CDI in adult patients the cure rate reaches up to approximately 100% and ranges from 87% to 90%

(29). FMT is also effective in recurrent CDI in the pediatric population (30).

The breakthrough in terms of FMT for CDI was the study described in 2013 showing that intraduodenal administration of stool-derived intestinal microbiota was effective in recurrent CDI in 81% of patients compared to oral vancomycin (31%) (20). It has been established that the efficacy of FMT in patients with multiple CDI relapses varies within 65-80% after a single application and 90-95% after repeated procedures compared to 25-27% for antibiotics (31). Similar results are obtained regardless of fresh or frozen material is used and irrespective of the route of administration. Admittedly, Ianiro et al. in a systematic review found that administration of fecal suspension (rectal infusion or colonoscopy) is more efficient than with the use of gastroscopy, nasogastric or nasojejunal catheter (84-93% *vs* 81-86% respectively) (29), other studies do not confirm the advantage of a specific route of administration (32) or method of infusion (in terms of the upper GI tract) (33), which suggests that in the absence of contraindications for oral administration, this route and capsule form may be preferred because of lower costs, simplicity and lower risk of complications associated with the procedure. A meta-analysis of Kassam et al. in patients with severe or recurrent CDI ≥ 500 cases) confirmed the high efficacy of FMT (87-90%) regardless of the route of administration (34). Kao et al. compared the effectiveness of FMT in the form of an oral capsule and stool suspension administered via colonoscope in the prevention of CDI recurrence (35). The percentage of patients without CDI recurrence 12 weeks after FMT was assessed in 105 patients. No difference in the effectiveness of FMT was disclosed. A significantly higher percentage of subjects receiving FMT in a capsule form rated FMT as "not at all unpleasant" (66% *vs* 44%). In general, randomized controlled studies have shown similar efficacy of frozen and fresh FMT for the treatment of CDI (36).

In the study by Duarte-Chavez et al. commercially available previously frozen stool samples of 250 mL volume, after thawing for 4 h administered as a suspension on colonoscopy proved to be effective in 86% of patients (37). Patients who failed to respond to FMT were older (70 *vs* 57 years), more often female (80 *vs* 67%), with severe CDI (80 *vs* 13%), and taking opioids (60 *vs* 37%).

Many clinical trials prove the advantage of sequential FMT *vs* single procedure in severe and severe/complicated CDI patients who are at high risk for colectomy (38, 39, 40). In Fishers et al. study the overall response of repetitive FMT via colonoscopy was 93% with 89% for severe/complicated CDI and 100% for severe CDI (38). Ianiro et al. found that severe CDI, just like suboptimal bowel preparation, were independent risk factors of failure after single fecal infusion (39) and confirmed in a subsequent randomized clinical trial that multiple FMT followed by vancomycin was more effective than a single fecal infusion and concomitant vancomycin in severe CDI not responding to antibiotics (100% *vs* 75%)(40).

Early FMT immensely reduces the 3-month mortality rate in severe CDI independent of age, sex, comorbidities, and ribotype of the *C. difficile* as disclosed in a retrospective cohort study of 111 patients hospitalized in the infectious diseases department (41). Worthy of note, Ianiro et al. confirmed a higher survival rate at 3 months in patients with recurrent CDI treated with FMT *vs* antibiotics, in addition to the lower incidence of bloodstream infections and 2 weeks shorter hospitalization (42).

It is not known what exactly underlies the high efficacy of FMT in CDI. *C. difficile* is an opportunistic bacterium. It is believed to cause disease in people with less intestinal microbiota biodiversity, usually caused by antibiotics that favor the growth of *C. difficile* (43). It has been observed that severe CDI is accompanied by a decrease in the number of *Lachnospiraceae* in the gut, and administration of the suspension containing these bacteria cures CDI in mice (44). In addition, *Bacillus thuringiensis* present in stool-derived transplant secretes bacteriocin (turicin) with *C. difficile* inhibitory activity (45). Other bacteria used in FMT have similar effects. Moreover, antibiotics increase the concentration of primary bile acids beneficial for the development of *C. difficile* (they promote the conversion of *C. difficile* spores into vegetative forms), whereas after FMT the concentration of secondary bile acids that adversely affect the reproduction of *C. difficile* increases (46).

It can be presumed that the mechanism of the beneficial effects of FMT is multifactorial and associated with the emergence of new bacteria in the host microbiota, an increase in the number of beneficial microorganisms present in negligible amounts in the patient's gut before FMT, displacement from the niche and competition for nutrients that creates unfavorable conditions for *C. difficile*. The renewal of *Firmicutes* and *Bacteroidetes* with an accompanying decrease in *Proteobacteria* may also be important (47). FMT leads to long-term colonization of new microbial species originating from the donor, as well as an increase in the population of own

microorganisms present in a reduced number before FMT.

Study of Petrof E.O. at al. shows that even a selection of fecal bacteria (composition of stool substitute of thirty-three isolates representing commensal species generally sensitive to a range of antimicrobials recovered from a healthy donor stool sample and abundant in *Eubacterium eligens, Eubacterium recital and Faecalibacterium prausnitzii*) used for FMT may be effective in the treatment of recurrent CDI caused by hypervirulent *C. difficile* strain (ribotype O78) (48).

Initially, recommendations for the use of FMT in the treatment of CDI differed from center to center (19, 37, 49, 50), but institutional, national (for example in Austria, France, the USA) or international consensus guidelines are available nowadays. Both the European Society of Clinical Microbiology and Infectious Disease and the American College of Gastroenterology recommend FMT for recurrent CDI (51, 52). Research on the use of FMT in the treatment of the first episode of CDI may influence guidelines in the future (53).

Inflammatory bowel disease

The etiopathogenesis of IBD is unclear and more complex than CDI involving, among others, abnormal immune response to the components of intestinal microbes. In IBD, a reduction in the population of *Bacteroidetes* and *Lachnospiracetae* (*Firmicutes*) and an increase in the number of *Actinobacteria* and *Proteobacteria* (54), as well as *Fecalibacterium prausnitzii* deficiency (in patients with Crohn's disease) was observed (55). Treatment of IBD for several decades has basically been limited to immunomodulation thus making FMT a promising therapeutic option for IBD patients. However, the results of FMT in IBD are worse than in CDI and do not allow to create guidelines at the moment, mainly due to the high heterogeneity of studies and their contradictory or ambiguous results. The effectiveness of FMT in IBD varies. A meta-analysis of 18 studies involving 122 IBD patients reported an average remission rate of 45%, which drops to 36.2% after excluding case reports (56). The remission rate in UC was 22% and in Crohn's disease 60.5%.

In a study by Rossen et al. 50 patients with mild to moderate UC who were given either suspension of feces from a healthy donor or autologous feces suspension as a placebo through a nasogastric tube show no differences in remission rates (clinical and endoscopic – reduction of disease activity by Mayo by at least 1) (57). In turn, Moayyedi et al. in 75 patients with mild or moderate exacerbation of UC used FMT in the form of rectal suspension or placebo (water) 6x at weekly intervals with a remission rate 24% in FMT subjects *vs* 5% in the placebo group (58). In the other study of patients with mild to moderate UC, a one-week treatment with anaerobically prepared donor FMT resulted in a higher rate of remission at 8 weeks (59).

Some believe that the research methodology rather than the concept itself influences the diversity of FMT results in IBD. Four randomized studies in UC have been performed, obtaining positive results in three of them – a higher percentage of clinical and endoscopic remissions compared to placebo (60). The study indicating the ineffectiveness of FMT was the only one in which intestinal microbiota was given through the upper GI tract. One study found that patients with the recent diagnosis of UC (= year) respond better to FMT, but other studies did not confirm the results. Worsening of IBD following FMT was also described (61).

It cannot be excluded that the result of FMT is largely donor-dependent (58). It has been reported that patients who respond to FMT show change of the host microbiota to the donor intestinal microbiota pattern and an increase of bacteria producing short-chain fatty acids. It is not known what exactly determines the success of FMT therapy. Is it the matter of specific bacteria and their metabolites or is it rather a consequence of the interaction between the microbiota and host-dependent factors? Patients who received a fecal suspension from a donor whose feces abounded in the *Lachnospiraceae* family and the *Ruminococcus* genus gained remission that corresponds to the results showing the *Lachnospiraceae* family deficiency in IBD (62). Based on a study of 272 patients, FMT was found to be less effective in treating CDI (at least two relapses after the first CDI episode) in patients with IBD compared to patients without IBD (74.4% vs. 92.1%) (63). Patients respond to FMT treatment similarly regardless of immunosuppressive therapy but, noteworthily, 25.6% of patients experienced an exacerbation of IBD after FMT.

Currently, FMT in IBD has the status of a method used in clinical trials and does not fall within the standard of IBD therapy, although FMT can be used to treat some cases of CDI in IBD patients.

Irritable bowel syndrome

Dysbiosis is one of the factors included in the etiopathogenesis of IBS. A common feature for CDI, IBD and IBS is the reduction of microbial biodiversity of the gut. In patients with IBS compared

to the healthy population, a higher amount of *Escherichia/Shigella* and *Aeromonas* and a reduction of *Acinetobacter*, *Citrobacter* and *Microvirgula* in the duodenal aspirate was found (64). In some IBS patients, dietary interventions decreasing bacterial fermentation in the intestines show good results. TARGET 1, 2, and 3 studies confirmed the efficacy of rifaximin in patients with non-obstructive IBS (65). Rifaximin, unlike other antibiotics, increases the amount of beneficial intestinal bacteria, hence it is referred to as eubiotic (66). Probiotics can also reduce discomfort and improve the quality of life of patients with certain functional disorders of the GI tract and fecal metabolites are associated with gut visceral sensitivity (67).

The therapeutic value of FMT in IBS remains unclear. The results of clinical trials and meta-analyses are inconclusive. Recently published results of the double-blind placebo-controlled study by El-Salhy et al., designed to evaluate the role of FMT in IBS treatment due to contradictory results of two previous randomized studies in this subgroup, proved the reduction in the IBS symptoms at 3 months after FMT in at least 76.9% of patients with additional improvement in fatigue and the quality of life (68). A thorough meta-analysis of five randomized controlled studies in 267 IBS patients suggests that fresh or frozen donor stool delivery through either upper GI tract (by nasojejunal tube) or lower GI tract (by colonoscope) may be beneficial in IBS (69). Contrary to Ianiro at al., a systematic review with the meta-analysis by Myneedu et al. published the same year (2019) did not confirm that FMT is effective in IBS (70). It is believed that heterogeneity of FMT protocols and the diversity of IBS patients may contribute to such ambiguous conclusions.

Obesity

Obesity has become an epidemic in developed countries. The intestinal microbiome of obese patients differs from lean people (71). Early antibiotic therapy promotes obesity in adulthood (72). The postulated basis for this phenomenon is rather not the fermentation of carbohydrates from food and the supply of additional calories as short-chain fatty acids, but the effect on the neuro-hormonal axis, energy expenditure, and a feeling of satiety. Studies in animal models indicate the beneficial effects of FMT in obesity. Intestinal microbiota transfer from the lean individuals increased insulin sensitivity, intestinal bacterial diversity and butyric acid synthesis in the obese hosts (73) and the evidence for the potential role of intestinal microbiota modulation in the treatment of metabolic syndrome is growing (74).

Other indications

A response to FMT has been described in a child with recurrent lactic acidosis resistant to standard treatment. This metabolic disorder occurs in people with short bowel syndrome when the intestinal bacteria produce excess D-lactate, which causes an increase in its concentration in the body, lactic acidosis and encephalopathy (75).

Numerous experimental studies in mammals indicate that dysbiosis-inducing factors can impair the central nervous system function, as intestinal microbiota affects cognitive function, behavioral patterns, social interactions, and stress responses. The results of studies in animal models suggest a beneficial effect of probiotics on behavior (76), which raises hope for the psyche modulation by the probiotic supplementation in humans. Perhaps in the future, such observation will also expand the list of indications for FMT.

Other potential indications for FMT include autism, type 2 diabetes, metabolic syndrome, fatty liver disease, hepatic encephalopathy, graft-versus-host disease, eradication of multi-resistant microorganisms or allergy, to mention at least some of them.

FMT tolerance and safety

Stool and its components currently do not have the status of medicine. Commonly acknowledged regulatory classifications for FMT have not been established yet in Europe (21). The European Medicines Agency either does not regulate the FMT status in the treatment of CDI. In the opinion of the European Commission, every member of the European Union has the right to individual decisions on FMT, however, there are no such regulations in most EU countries. Nevertheless, some countries have issued national rules and others require to be compliant to the European directive 2004/23 on quality and safety of tissues and cells as FMT meets the criteria of "substance of human origin" category. Therefore, knowledge about tolerance and complications of FMT comes almost exclusively from case reports, clinical studies and relatively few meta-analyses that suggest that FMT is a safe and well-tolerated procedure. In the study by Duarte-Chavez et al., loose stools were the most commonly reported FMT adverse reactions (37). In Meyers et al. study the incidence of mild side effects (mainly abdominal discomfort) was 30%, and severe complications such as severe infections (0.7%) and death (0.1%) were rare (31). In IBD patients the pro-

portion of minor adverse events was 5.4% in the group of patients in whom the intestinal microbiota was given orally as capsules and 12.5% in the group in which the stool suspension was administered by a colonoscope (35).

It should be remembered that the relative simplicity and widespread availability of biological material for FMT prompts more desperate patients to attempt to perform this procedure at home without taking into account any recommendations. They constitute the "gray area" of FMT safety profile assessment.

Possible undesirable effects of FMT are divided into early (dependent and independent on the procedure, mild and severe) (Table 2) and late, of which currently little is known. Procedure-related complications are e.g. endoscopy complications, such as GI perforation or bleeding or associated with possible analgosedation during the procedure, e.g. aspiration of content into the respiratory tract.

Severe early complications independent of the procedure are very rare. Two cases of norovirus infection diagnosed 2 and 12 days after FMT (77) and bacteremia caused by *E. coli* a day after FMT in a patient with IBD who had such episodes previously preceding FMT were described. Usually, even in high-risk subjects, e.g. immunosuppressive patients treated for IBD no serious complications clearly attributed to FMT are observed.

The value of FMT in the treatment of CDI is well documented, but long-term adverse effects are unknown (78, 79). Possible late complications of FMT include the risk of transferring pathogens and the induction of diseases with etiopathogenesis related to the intestinal microbiota. The hypothetical list seems to extend: IBD, IBS, microscopic enteritis, fatty liver disease, atherosclerosis, obesity, diabetes mellitus, asthma, autism, rheumatoid arthritis, Sjögren's syndrome, idiopathic thrombocytopenia, peripheral neuropathy, and weight gain.

SUMMARY

Recently, much attention has been paid to the role of intestinal microbiota in health and disease. FMT is one of the methods to restore eubiosis. The best proven indication for FMT are cases of CDI not responding to standard treatment. As the research progresses, the list of potential indications for FMT increases, although sometimes based on limited data. Well-planned studies on large groups of patients are needed.

The undoubted advantage of FMT is generally good tolerance of this method, the possibility to use in outpatient settings and acceptable costs of the procedure compared to pharmacotherapy or surgical treatment. Despite these advantages, FMT is still a procedure with limited availability. It seems that potential patients are more interested in FMT than some clinicians, perhaps due to dissatisfaction of patients with current treatment methods and, in the case of medical staff, unknown distant complications and the lack of legal regulations regarding FMT. Other obstacles to the widespread use of FMT are aesthetic considerations, sometimes invasive endoscopic nature of intestinal microbiota transfer and, thus, greater financial outlays. These barriers may disappear with the increased availability of oral capsules.

It is likely that FMT will become more popular in western countries, partially due to growing interest in "natural" methods of treatment. The emergence of stool banks and the availability of convenient oral capsules would facilitate the application of FMT on a larger scale in clinical trials and everyday practice. The development of referral FMT centers

Table 2. Early complications in the course of FMT (12).

Early FMT complications	
Mild complications	**Severe complications**
Abdominal discomfort	
Colic abdominal pain	
Flatulence	Infection with intestinal pathogens
Diarrhea	Bacteraemia
Constipation	Sepsis
Borborygmi	Peritonitis
Nausea	Pneumonia
Vomiting	Exacerbation of IBD
Belching	
Transient fever	

with appropriate expertise and facilities as well as stool banks is strongly encouraged.

It can be assumed that FMT is a kind of "bridge" therapy. With the research progress, it may be possible in the future to use only selected strains or active compounds isolated from the feces of healthy donors sold like any other pharmaceutical dosage forms of drugs at the pharmacy. The first attempts in this direction are already being made.

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Conflict of interests

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