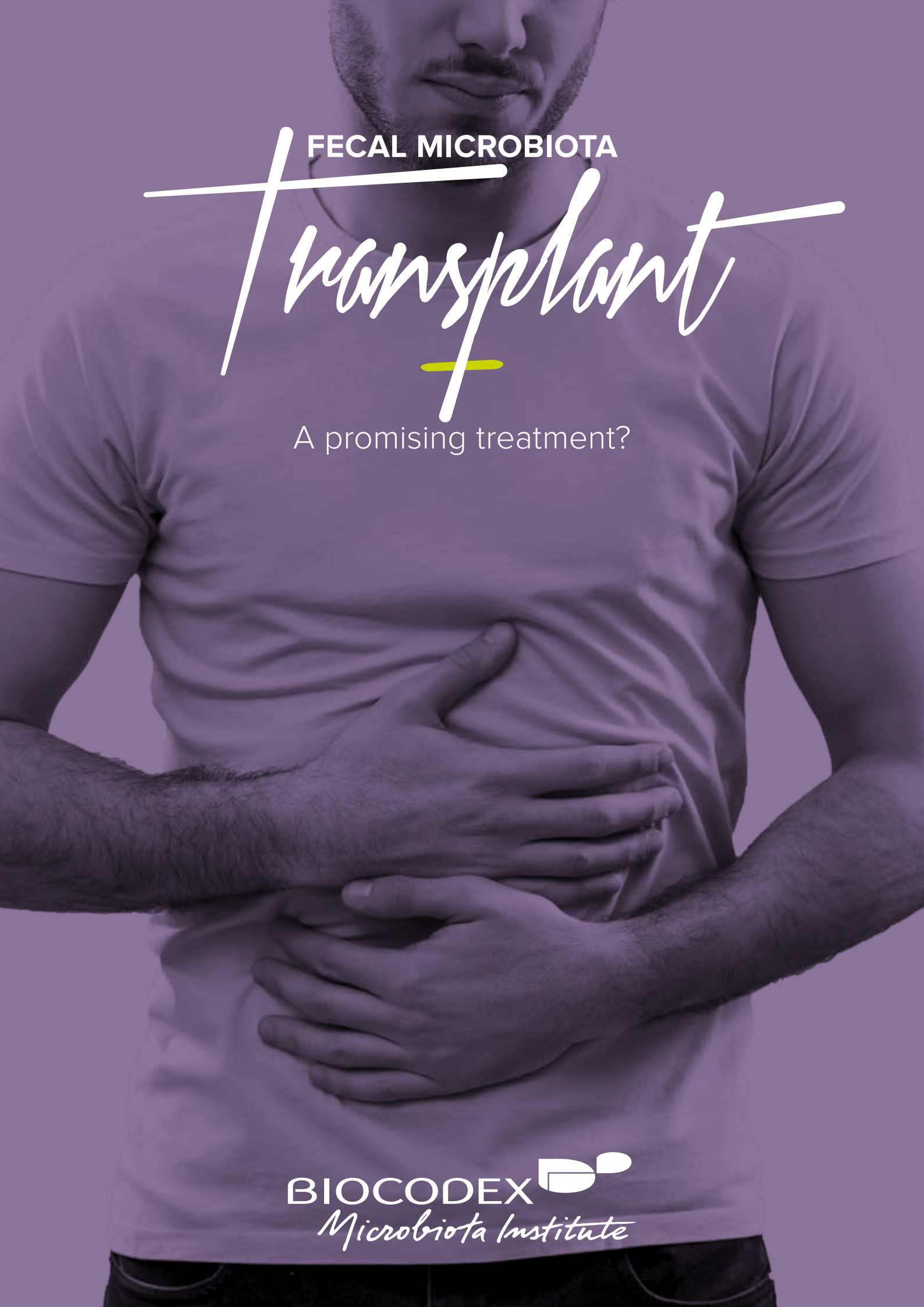

FECAL MICROBIOTA transplant

A promising treatment?

BIOCODEX
Microbiota Institute

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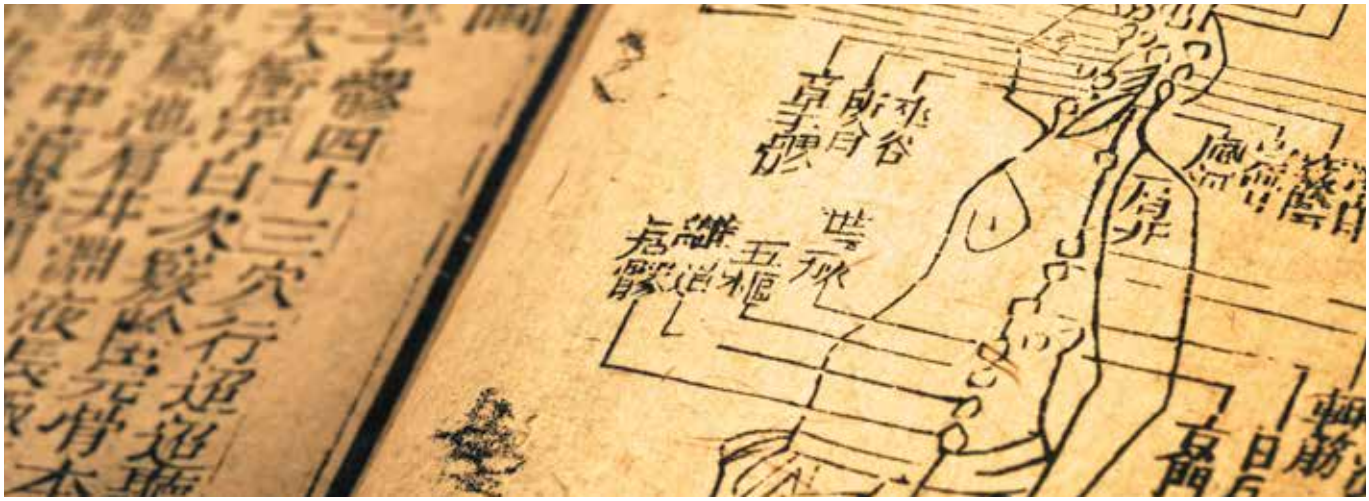
1 • WHAT IS FMT?



Although the media only recently became interested in this topic, fecal microbiota transplant is actually a very old medical practice. Its history goes all the way back to 4th-century China. It was then called "yellow soup" and was used to treat diarrhea and food poisoning. But the first experiment with a fecal enema was only conducted in 1958. In 2013, 45 years later, the results of the first clinical trial on FMT were published, demonstrating that it is superior to antibiotics in the treatment of recurrent *Clostridium difficile* infections[1], a bacterium which is responsible for 20 to 30% of antibiotic-associated diarrhea that can sometimes be severe. It was the start of the craze towards FMT, first from researchers and later from the general public.

The mechanism of FMT: transplanting to restore the balance

Fecal microbiota transplant (FMT), also known as stool transplant, is an original therapeutic approach that aims at restoring gut microbiota balance. It consists in administering microorganisms found in the stools of a healthy donor to restore the gut microbiota of a patient suffering from a disease associated to a disruption in the gut microbiota. Although FMT has only one approved indication so far–i.e. the treatment of recurrent *Clostridium difficile* colitis–the research is now focused on other therapeutic avenues: some gastrointestinal disorders, autism, obesity, depression, etc., with mixed results[2]. How is FMT regulated? What are its potential future uses and its limits?

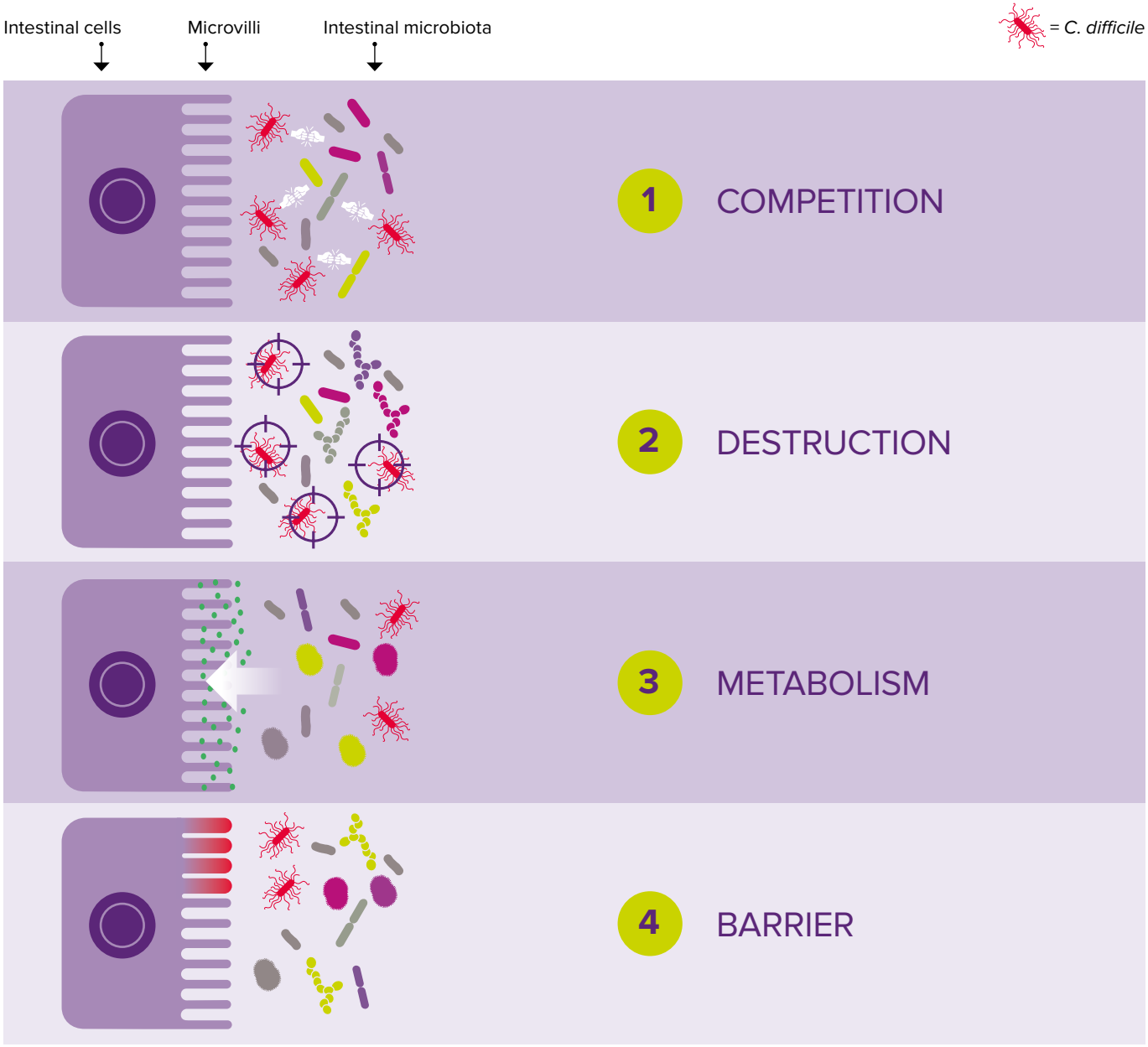
The gut microbiota (or gut flora) is an organ in its own right, made of billions of microorganisms (bacteria, fungi, viruses…) constantly interacting with each other as well as with the organism they are colonizing (host). In the colon, microorganisms fiercely compete for available space and food, but they also work closely together to digest large molecules. As a treatment for *Clostridium difficile* infection, FMT is based on four mechanisms of action[3]:

1 A relapse of *C. difficile* infection is defined by the return of symptoms associated to this bacterium and the presence of its toxins in patient's stool within 8 weeks following the end of a well-conducted treatment and without further antibiotic treatment that could have triggered it (Source: Sokol H. Transplantation fécale. Post'U(2018))

2 Lagier JC, Raoult D. Fecal microbiota transplantation: indications and perspectives. Med Sci (Paris). 2016 Nov

3 Khoruts A, Sadowski MJ. Understanding the mechanisms of faecal microbiota transplantation. Nat Rev Gastroenterol Hepatol. 2016 Sep;13(9):508-16. doi: 10.1038/nrgastro.2016.98

The TMF four mechanisms of action



1 **Competition for available space and food:** by reintroducing the microorganisms that were destroyed by antibiotics, it is believed that FMT restores the competition for resources and space: as *C. difficile* would have decreased space and nutrients, its growth would thus be limited;

2 **Destruction of the pathogen:** by restoring the bactericidal or bacteriostatic action (i.e. destroying bacteria and limiting their growth, respectively) through the supply of "killer" bacteria, FMT helps eradicate *C. difficile*;

3 **Restoration of the normal host's metabolism:** the destruction of microorganisms by antibiotics leads to changes in the metabolism of bile acids and provides a favorable environment for the colonization by *C. difficile*; secondary bile acids from the healthy donor are intended to prevent the growth of *C. difficile*;

4 **Restoration of the intestinal barrier function:** by providing elements that are necessary to the regeneration of the inner lining of the gut (epithelium) and to the production of antimicrobial molecules, FMT could restore the gut barrier and mitigate the body's inflammatory reaction to *C. difficile*.

FMT, in practice: from the donor to the side effects

We can easily imagine how skin, kidney or lung transplants are performed. But what about fecal microbiota transplant (FMT)? Faced with the explosion of new research on FMT, health authorities from several countries (including France) published recommendations aiming at regulating this practice, and especially donor selection, because a transplant requires a donor, and not anyone is eligible to donate their stool!

Is fecal microbiota a drug?

The answer depends on the country. In France and in the US, fecal microbiota is considered as a drug. It is not the case in the UK, Denmark or the Netherlands. In France, the National Agency for the Safety of Medicine and Health Products (ANSM) published in March 2014 and updated in 2016 a document regulating FMT which describes the procedure, especially the donor selection process.

4 La transplantation de microbiote fécal et son encadrement dans les essais cliniques. ANSM. Novembre 2016 (actualisation de la version de juin 2015) https://ansm.sante.fr/content/download/79197/1003045/version/3/file/Microbiote-fecale-rapport-nov-2016.pdf

A RIGOROUS DONOR SELECTION PROCESS

As with any transplant, the donor must be chosen according to very strict criteria in order to minimize the risk of transmitting an infection or another disease.

1st PHASE

18 years old

It is recommended to be under 65 years old, but age is not an exclusion criterion.

Blood relations

The donor can be a blood relative of the recipient and anonymity is not required. Specialists believe they should be allowed to ask for relatives' help provided they are perfectly healthy[5].

Medical exam as well as blood and stool tests[6]

Objective: make sure that there is no contraindication to the donation, such as chronic diseases, excess weight (body mass index (BMI) above 30), gastrointestinal disorders, long-term drug treatment, hospitalization in a foreign country that lasted over 24 hours in the past year, or a recent or long-term stay (several years) in a tropical region; and to look for the possible presence of viruses (HIV, hepatitis viruses, cytomegalovirus), bacteria (*C. difficile*, Salmonella, Listeria...) or parasites (*Toxoplasma gondii, amoebae, Giardia intestinalis*...).

Pre-screening questionnaire

The prospective donor must fill out a very detailed pre-screening questionnaire regarding their medical history and lifestyle to determine their microbiota composition (diet, tobacco use...).

Potential donors

2nd PHASE

The final selection only takes place after the second medical interview is performed and a short questionnaire on events occurred since the pre-screening visit is completed, just before the donation[3].

Short questionnaire

2nd medical exam

Specific procedure

In France, FMT must be prepared under the responsibility of the in-house pharmacy of a health facility[3]. The collected stools are diluted, mixed, filtered and then filled into syringes before being administered. They can also be frozen, which provides the opportunity to create stool banks that are available at any moment[5]. The ANSM adds that "freezing could also limit the risk of transmission of infectious agents and bypass the pre-screening step (the screening could then be carried out on the transplant itself".

5 Cammarota G, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017

6 https://www.snfge.org/content/la-transplantation-de-microbiote-fecal-tmf-da

Several routes of administration

Through upper route introducing a tube into the nose of patients and reaching the stomach (nasogastric tube) or the duodenum (nasoduodenal tube).

Through lower route using an enema or colonoscopy. In the latter, a colon cleansing must be carried out prior to the injection of the preparation[2]. Some medical teams use fecal matter capsules: 2 x 15 capsules administered as two doses separated by a 24-hour interval[7].

VERY LIMITED ADVERSE EVENTS[5]

Adverse events of FMT are usually moderate and, for the most part, of gastrointestinal nature. They occur within hours following the transplant and disappear within 48 hours:

- **diarrhea** in **75%** of patients,
- **abdominal pain** in **50%** of them,
- more rarely, **constipation**.

Severe adverse effects are extremely rare, but they are enough to warrant a strict donor selection process: bacteremia, norovirus infection (two published cases), increased weight (one reported case), acute pulmonary edema (one reported case). Some are related to the administration route, for instance gastrointestinal perforations.

7 Modalités pratiques pour la réalisation d'une transplantation de microbiote fécal (TMF). Groupe français de transplantation fécale. https://www.gftf.fr/45+modalites-pratiques-pour-la-realisation-d-une-transplantation-de-microbiote-fecal-tmf.html

2. PROVEN EFFICACY FOR A SINGLE INDICATION

Currently, fecal microbiota transplant (FMT) is only indicated for the treatment of recurrent *Clostridium difficile* infections, but its indications might soon include other diseases where the involvement of the gut microbiota has been confirmed.

Recurrent *Clostridium difficile* colitis: the only validated indication

Recurrent *Clostridium difficile* colitis is the first cause of treatment-related diarrhea and affected over 450,000 Americans in 2011, of which 30,000 died. In France, 1800 deaths were associated to *C. difficile* infection in 2014[2]. An estimated 5% of overall mortality in hospitalized patients is attributable to this bacterial strain.

Until the 1990s, *C. difficile* colitis was a relatively rare infection which was not considered a health hazard: an antibiotic treatment was enough to get rid of it. But in a 20-year period, the frequency of this disease more than doubled, while the efficacy of the antibiotics therapy dropped to a 20-30% success rate[8,9]. The bacterium is becoming increasingly resistant to antibiotics. It was not until the start of the 2000s and the sequencing of *C. difficile* genome when a particularly virulent strain was identified. It is resistant to antibiotics and able to produce 10 times the amount of toxins usually secreted by this bacterium.

8 Moayyedi P, et al. Faecal microbiota transplantation for *Clostridium difficile*-associated diarrhoea: a systematic review of randomised controlled trials. Med J Aust. 2017

9 Wortelboer K, et al. Fecal microbiota transplantation beyond *Clostridioides difficile* infections. EBioMedicine. 2019 Jun.

Beware of relapse with repeated antibiotic courses!

Infection generally occurs after the destruction of the gut microbiota by repeated courses of antibiotics. *C. difficile*, which is present as resting spores in the colon, proliferates and changes to produce toxins that cause inflammation and diarrhea. Paradoxically, this infection is treated with antibiotics, which progressively exacerbate the gut microbiota disruption at each additional treatment course[3] thus leading to a 35% rate of relapse[2].

FMT should be preferred to antibiotics for recurrent cases

In 1958, the surgeon Ben Eiseman published 4 cases of pseudomembranous colitis that were cured with FMT and sparked interest in this method. Several articles described its efficacy to treat the recurrent form of this disease. But the true turning point came in 2013 with the publication of the first clinical trial in humans. This trial was designed with a robust methodology and demonstrated the therapeutic superiority of FMT over antibiotic therapy to treat recurrent and drug-resistant forms of *C. difficile* infections.

INTERNATIONAL RECOMMENDATIONS FOR THE TREATMENT OF *C. DIFFICILE* INFECTIONS[10]

- Following the publication of the Dutch study, the European Society of Clinical Microbiology and Infection (ESCMID) updated its recommendations and included FMT as a possible treatment for recurrent *C. difficile* infection.

An isolated severe episode or a first colitis relapse must be treated with oral antibiotics.

Only the second relapse, which characterizes recurrent *C. difficile* colitis, warrants the use of a stool transplant.

10 https://onlinelibrary.wiley.com/doi/full/10.1111/1469-0691.12418

11 Sokol H. Transplantation fécale. Post'U(2018)

3. WHAT ABOUT TOMORROW?

Although *Clostridium difficile* colitis is the only approved indication for the use of fecal microbiota transplant (FMT), all diseases for which the gut microbiota is believed to play a role have drawn the interest of scientists. Here is an overview of the current state of research on FMT, from gastrointestinal diseases to cancer, all the way to neurological disorders.

Chronic inflammatory bowel diseases

Chronic inflammatory bowel diseases (CIBD) are characterized by the inflammation of the gastrointestinal wall. In Crohn's disease (CD), the damage can reach the entire gastrointestinal system, from mouth to anus, but it is usually more localized to the terminal part of the small intestine and the colon. In ulcerative colitis (UC) it is limited to the colon and rectum[12].

CIBDs progress as inflammatory flare-ups of variable duration and frequency depending on the patient, alternating with periods of remission. They cause acute abdominal pain, severe diarrhea (between 5 and 10 bowel movements per day), associated with blood and pus in ulcerative colitis (UC); and in severe forms, other symptoms and complications can occur such as fever, tachycardia, nausea and vomiting, weight loss and dehydration. CIBDs also trigger extra-intestinal symptoms, especially articular pain, skin and mucosa lesions (skin ulcerations, mouth ulcers, glossitis, i.e. tongue inflammation...) as well as hepatic and ocular disorders[13].

12 Pierre Desreumaux (Unité Inserm 995). Maladie inflammatoires chroniques de l'intestin. Inserm. 2016

13 Maladies inflammatoires chroniques de l'Intestin (MICI). Inserm. Fév. 2016 https://www.inserm.fr/informationen-sante/dossiers-information/maladies-inflammatoires-chroniques-intestin-mici et http://marker.to/wbVgfq

Unbalanced microbiota

The gut microbiota analysis of patients with UC revealed they had a decreased diversity of microbial species[14], especially a lower content of *Firmicutes* and *Bacteroidetes*. But it is mainly the low content of *Faecalibacterium prausnitzii* and the excessive content of Proteobacteria and Actinobacteria that are associated with these CIBDs. This imbalance causes a decrease in the production of short-chain fatty acids, beneficial substances which are food to colon cells and play an important role in the regulation of the immune system. That is why fecal microbiota transplant (FMT) has been considered to treat this disorder.

Moderate benefits in UC

Three of the four published clinical trials on UC concluded that this approach was beneficial. Overall, the beneficial effects were much more moderate than they were in the treatment of *C. difficile* colitis and depend on the donor–which is why donor selection is so important. And several results raise new questions: are only some microorganisms efficient? And if so, which ones? Should the patient receive an enema or an antibiotic therapy beforehand? Which administration route is preferable? Does the restoration of the gut microbiota work long-term or must transplants need to be repeated? These questions need to be answered before fecal microbiota transplant can be seriously considered as an alternative for the treatment of ulcerative colitis.

14 D'Haens GR, Jobin C. Fecal Microbial Transplantation For Diseases Beyond Recurrent *Clostridium Difficile* Infection. Gastroenterology. 2019 June

Functional gastrointestinal disorders

There are many names for these diseases: irritable bowel syndrome (IBS), spastic colitis, colopathy or even functional gastrointestinal disorders (FGID). However, it is not just a semantic issue. In all cases, the quality of life of the patient is significantly impacted.

The microbiota of patients with irritable bowel syndrome is less diversified and shows greater abundance of enterobacteria, but with a lower content in bifidobacteria and lactobacilli. Functional gastrointestinal disorders are characterized by a decreased production of butyrate and an increased production of acetic and propionic acids, which are three substances associated with bloating. Abdominal pain, diarrhea and constipation are other symptoms of these disorders. In the US, an estimated 20% of the population is affected[14].

Controversial effects

Aside from ulcerative colitis, irritable bowel syndrome is the only other gastrointestinal condition for which a treatment with fecal microbiota transplant has been studied in clinical trials[14]. One of them showed a decrease in intestinal discomfort, abdominal pain and flatulence in transplant recipients. However, the results varied depending on the initial nature of the gut microbiota: the best responders to fecal microbiota transplant had an initial high content of Streptococci and they had a greater increase in biodiversity. Other works confirm the increased diversity and abundance of the microbiota after the administration of fecal matter capsules; however, patients reported a better improvement in symptoms when they received… placebo! Although these results do not undermine the efficacy of FMT in people with functional gastrointestinal disorders, detailed microbiota analyses before and after the transplant are necessary, according to the researchers.

What about constipation?

Constipation is potentially associated to gut dysbiosis and has been the focus of several works aiming at assessing the usefulness of FMT in this transit disorder[13]. In a study conducted in about sixty adults suffering from slow transit that compared the standard treatment to 6 courses of FMT, the latter was shown to cause a significant improvement of symptoms and transit, and more generally, in the quality of life[15]. These encouraging results still need to be confirmed: studies are also ongoing with specific strains, lactobacilli and bifidobacteria[13].

15 Tom Holvoet, et al. Fecal Microbiota Transplantation in Irritable Bowel Syndrome with Predominant Abdominal Bloating: Results from a Double Blind, Placebo-Controlled Clinical Trial. Gastroenterology. 2018

Hepatic encephalopathy

Hepatic encephalopathy is a major complication observed in patients with liver disease (cirrhosis) and is characterized by neurological disorders: cognitive disorders, personality changes or confusion. The treatment is based, among others, on the use of antibiotics which progressively alter the gut microbiota at every treatment course. As a result, many relapses occur that eventually cause irreversible damage to the brain.

The presence of a gut dysbiosis—characterized by reduced levels of beneficial SCFAs-producing bacteria and increased levels of harmful bacteria associated to cognitive disorders—was observed in patients with hepatic encephalopathy. These observations led researchers to consider fecal microbiota transplant as a therapeutic alternative to antibiotics[16].

A unique donor

An initial study focused on this indication was conducted on a small cohort of only twenty cirrhotic men, who were administered either the standard treatment, or FMT combined with an antibiotic pretreatment aiming at preparing the recipient's digestive tube. The fecal microbiota came from one single donor, selected through an AI software based on the abundance in his/her microbial flora of bacteria that are precisely lacking in these patients.

A promising approach

None of the transplant recipients had an additional episode of encephalopathy, while 5 out of the 10 control patients relapsed. A slight increase in lactobacilli and bifidobacteria was also observed in the former, while no change was observed in the latter. Finally, only FMT produced an improvement in cognitive functions which led researchers to advocate for further investigation.

Metabolic disorders

Diabetes mellitus, hypertension, cardiovascular diseases... Drugs have shown their limitations in the treatment of these diseases that are associated to excess weight and sedentary lifestyle.

In animals, the physical trait "obese" or "thin" can be transferred through fecal microbiota transplant with a clearly established causal link. In humans, the situation is a little more complex but the presence of dysbiosis associated to metabolic disorders in obese or hypertensive subjects led scientists to conclude that FMT could be a promising avenue. Works are ongoing to assess the impact of the change in gut microbiota experienced by patients with metabolic syndrome[17,14].

16 Bajaj JS, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. Hepatology. 2017

17 Metabolic syndrome, or syndrome X, refers to a cluster of physiological and biochemical disorders of lipid, carbohydrate or vascular origin associated to excess weight (French Federation of Cardiology) https://www.fedecardio.org/Les-maladies-cardio-vasculaires/Les-pathologies-cardio-vasculaires/zoom-sur-lesyndrome-metabolique)

Mixed benefits

Several clinical studies were conducted on obese patients with a metabolic syndrome. The first one was carried out on a small group of individuals and showed that stool transplant from thin donors improved the metabolic profile of recipients. The second included a larger number of patients and produced more mixed results. Only a few participants had an improved metabolic profile after the FMT, namely those who initially had a gut microbiota that was not very diversified. The response to transplant thus seems to be dependent on the patient's initial gut microbiota. However, the benefits did not withstand the test of time...nor did the transformation of the gut microbiota, which quickly returned to its initial composition.

Complex relationships

Overall, these results highlight the complexity of the link between the gut microbiota and metabolic functions. According to some scientists[13], metabolic and microbial responses to FMT could be based on interactions between the donor's microbiota and the recipient's. Several trials are ongoing to assess this technique's ability to reduce metabolic disorders as well as several obesity-related parameters. These eagerly awaited results should open the way to new strategic approaches in the treatment of metabolic syndrome.

STOOL TRANSPLANT: A PROMISING SOLUTION AGAINST ANTIBIOTIC RESISTANCE?

Antibiotic resistance keeps growing as a result of gut's colonization by microorganisms which have become insensitive to antibiotics. Could the solution be a fecal microbiota transplant? Several studies support this hypothesis[19].

By triggering a competition within the gut microbiota, FMT leads to the decolonization of several bacteria that are resistant to different antibiotic families (*Escherichia coli* resistant to cephalosporins, *Enterococcus* resistant to vancomycin, o enterobacteria resistant to carbapenems). In a clinical trial comparing antibiotic therapy followed by FMT with no intervention at all, the former was more successful (41% of patients were "decolonized" vs. 29% respectively)[20]. In two prospective studies where FMT was used–in one by itself and in the other together with an antibiotic pretreatment–it proved twice as much successful (up to 88%) to reduce populations of resistant bacteria[8].

If ongoing trials, including a larger number of patients, confirmed these excellent results, FMT could contribute to solving a major health scourge.

18 Kootte RS, et al. Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. Cell Metab. 2017

19 Davido B, Batista R, Fessi H et al. Fecal microbiota transplantation to eradicate vancomycin-resistant enterococci

20 Huttner BD, et al. A five-day course of oral antibiotics followed by faecal transplantation to eradicate carriage of multidrugresistant Enterobacteriaceae: A Randomized Clinical Trial. Clin Microbiol Infect. 2019

Professor Harry Sokol is a hepato-gastroenterologist at the Saint-Antoine Hospital (Paris, France). He is also the chairman of the French Group of Fecal Microbiota Transplant (GFTF). Though he understands why this therapeutic approach–still in its infancy–is gaining popularity, he explains the obstacles it is facing.

Fecal microbiota transplant: a miracle cure?

The frenzy surrounding fecal microbiota transplant (FMT) is real and it needs to be slowed down a bit: some patients have unrealistic expectations regarding the benefits of FMT in their particular case. Every week I receive dozens of letters about anything and everything. However, FMT is a not a magical cure! For now, it is indicated to treat a single disease: recurrent *C. difficile* infection. For all other indications, it is just a potential therapeutic avenue which cannot replace current treatments. Moreover, the future most likely lies in treatments combining stool transplant (or other therapies that target the microbiota) and more standard treatments that target the immune system, for instance.

Why does *C. difficile* respond that well to fecal microbiota transplant?

This infection is almost exclusively related to a disruption in the gut microbiota, while in other diseases, the role of the gut microbiota–although presumed–is only one of several contributing factors and its importance probably varies significantly from one disease to another. For instance, in the case of ulcerative colitis, for which we have the strongest data: clinical trials indicate a 20 to 30% rate of remission within a 8-12 week period; which is good, but very far from the results obtained in *C. difficile* infections (near 90%). This clearly shows that other factors (immune, genetic...) also play a role.

> **“FOR NOW, FMT IS INDICATED TO TREAT A SINGLE DISEASE: RECURRENT *C. DIFFICILE* INFECTION”**

Are there obstacles to the development of clinical research focused on FMT?

Research on FMT is still at a very early stage since it started less than 10 years ago; which is why we must take the time needed to assess it properly. In France, the handling of feces is subjected to major regulations and the selection of donors is strictly regulated. As a result, clinical trials are expensive and require complex logistics. In addition, since hospitals do not automatically assign a budget to FMT, the mobilization of health professionals varies from one facility to another, thus depriving researchers from a specific structure to rely on. It is time for public authorities to better understand this issue and to invest in order to provide hospitals with the means to develop this line of research. In *Assistance Publique-Hôpitaux de Paris* (Paris public hospital system), we hope to quickly see the emergence of a structured approach to FMT in the healthcare system.

FECAL MICROBIOTA transplant

The principle of fecal microbiota transplant (FMT)–also called stool transplant–is to administer microorganisms found in the stools of a healthy donor to a patient (i.e. the recipient) in order to restore their gut microbiota balance.

Efficacy of FMT has only been successfully demonstrated for a single disease (recurrent *Clostridium difficile* colitis) but researchers are now focused on other therapeutic avenues. All diseases for which the gut microbiota is believed to play a role are drawing the interest of scientists: gastrointestinal diseases, neurological disorders such as autism, obesity, depression, etc.

Although the media only recently became interested in this topic, FMT is actually a very old medical practice. Its history goes all the way back to 4th-century China. How is FMT regulated? What are its potential future uses and its limits? Here is an overview of the current state of research on FMT.

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