The application of fecal microbiota transplantation in patients with oncologic diseases

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Praca została wykonana w ramach projektów WNM SUM KNW-1-061/K/8/O oraz KNW – 2- O43/D/8/N.

Abstract: Oncologic patients often undergo a long-term hospitalization and exhaustive treatment. For this reason they often suffer from intestinal microbiota violations and dysbiosis, which results in C. difficile colonization and infection. Disorders in the composition of microbiota also can predispose to the development of some cancers. There are more than 2,000 bacterial species in the human intestine, with the composition of microbiota changing during a life. These microorganisms, often called “colonization resistance factor” are an important barrier for infections, caused by intestinal pathogens. Fecal microbiota transplantation (FMT, also referred as stool transplantation, fecal transplantation, fecal flora reconstitution, or fecal bacteriotherapy) is a procedure involving the transfer of a specially prepared stool sample from a healthy donor to the recipient, suffering from intestinal dysbacteriosis. The purpose of FMT is to rebuild and restore the normal bacterial microflora, especially the population of anaerobic bacteria. Currently, FMT is used for treatment of the recurrent C. difficile infection (rCDI), as well as other diseases related to the gastrointestinal tract, such as Inflammatory Bowel Disease, neurological, hematological and functional disorders. The stool donor may be related or not with the recipient, but most importantly, each donor should undergo many examinations similar to those for organ donors. Besides these, the presence of multi-resistant bacteria in the feces as well as a number of etiological agents of intestinal infections (bacteria, viruses and parasites) should be excluded from donatation. On the other hand, the FMT procedure as an application of biologically-alien material seems to be quite risky, especially in patients who are undergoing anticancer and immunosuppressive therapy. However, in most cases, when FMT has been used, positive therapeutic effects for eradication of CDI have been described. Moreover a protective action against acute graft-versus-host disease has been observed. In this review, we will focus on the problem of FMT usage in oncologic patients, in the light of recent publications.

1. Introduction

FMT (fecal microbiota transplantation, but also referred to as stool transplantation, fecal transplantation, fecal microbiota reconstitution, or fecal bacteriotherapy); is a procedure involving the transfer of a stool sample from a healthy person, to the recipient who has disturbed composition of the intestinal microbiota. These disorders also are called bacterial or intestinal dysbiosis which are abnormalities in quantitative and qualitative composition of commensal microbiota (Hill, Hoffman et al. 2010). Dysbiosis may lead to the development of inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), diabetes, obesity, cancer, cardiovascular and central nervous system disorders (Belizario, Faintuch 2018). The purpose of FMT is to reconstruct and restore normal bacterial population of the recipient’s intestinal microbiota. Currently, FMT is used for the treatment of recurrent Clostridoides difficile infection (rCDI). Besides rCDI treatment, FMT is also used in other gastrointestinal (GI) inflammatory diseases, such as Inflammatory Bowel Disease and non-gastrointestinal diseases (n-GI). There are attempts made to use FMT in the treatment of: autism, chronic fatigue syndrome, fibromyalgia, idiopathic thrombocytopenic purpura, multiple sclerosis, myoclonus dystonia, and Parkinson disease. However lack of randomized trials makes it impossible to develop strict guidelines for the treatment of these diseases (Vindigni, Surawicz 2017).

The number of microorganisms inhabiting the human intestine is up to 1014/ml of feces. Based on the bacterial analysis of 16S rRNA, 2172 bacterial species were identified, of which 386 were classified as obligate anaerobes. Anaerobes are classified in 12 different phyla, of which 93.5% belonged to Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes (Thursby, Juge 2017, Hill, Hoffman et al. 2010). The composition of the physiological microbiota inhabiting the intestine changes over the course of life. The intestine of newborns after delivery is quickly settled with bacteria. Type of bacteria depends on the type of delivery and the method of feeding. The intestine of newborns with natural delivery is quickly settled mainly with Lactobacillus spp., while those after Caesarean section is mainly colonized by Bacteroides spp. and Clostridium spp. During the first year of life the diversity of intestinal microbial species increases, so that after the child re-
aches 2.5 years, it has a microbiota characteristic for an adult. The composition of this microbiota may also vary depending on diet, accompanying diseases or medication. Subsequent changes occur after the age of 65, when again the number of *Clostridium* and *Bacteroides* genus increases. With the aging of the body, rearrangement in the intestinal microbiota, leads to an increase in butyric acids production and a decrease in short-chain fatty acids production as well as the decrease efficiency of the process amylolysis (Thursby, Juge 2017, Quigley 2017, Arumugam, Raes et al. 2011, Bhatt, Redinbo et al. 2017). Intestinal microbiota is responsible for a large variety of important functions and processes in human life, such as:

- induction of synthesis of antimicrobial particles increasing of mucin gene expression, increasing of IgA secretion

**2. Search strategy and selection criteria**

The aim this study was to find an answer to the question whether the FMT procedure applies in the course of treatment of oncological patients? In order to achieve this goal, the literature was reviewed in the PubMed, Google Scholar, and Medline databases by entering: Fecal microbiota transfer, Fecal microbiota transplantation and cancer, Fecal microbiota transplantation and oncology, *Clostridium difficile* infection and cancer, *Clostridium difficile* infection and oncology. Particular attention was paid to English-language articles from recent years. The search revealed a large number of publications on the FMT procedure and its use in the treatment of intestinal diseases, while information on the use of FMT in patients undergoing oncological treatment is scarce.

**3. State of the Art**

**FMT History**

An intestinal microbiota transplantation is not an invention of our time. In ancient China, in the fourth century, attempts were made to treat food poisoning and severe diarrhea by using stool administered to the patient’s mouth. The Chinese manual “Handy Therapy for emergencies” said that this therapy is wonderful and brings patient back from the edge of death. In the 16th century, various fecal preparations (fermented fecal solution, fresh fecal suspension, dried feces, feces from infants) were named by the doctors of alternative medicine “yellow soup” or “golden syrup”. Li Shizhen described their use in the treatment of severe diarrhea, fever, pain, vomiting and constipation. In the seventeenth century, the Italian doctor Acquapendente tried to apply sick animals an intestinal microbiota transplant from healthy animals basing on the observed coprophagy in animals. In the nineteenth century there was a breakthrough when Antonie van Leeuwenhoek discovered bacteria in the stool. Another scientist was Russian zoologist Ilja Miecznikow, who observed among poor Bulgarian farmers maintaining good health, thanks to drinking fermented milk, and then linked the occurrence of *Lactobacillus bulgaricus* in milk, as a protective and health-improving bacterium. German physician and bacteriologist Alfred Nissle isolated *Escherichia coli* and noted that *Escherichia coli* competes with *Shigella* spp. for growth factors. During World War II, German soldiers in Africa used a Bedouin way against dysentery - eating fresh, warm camels stool. Nazi scientists isolated from this stool *Bacillus subtilis*, which was later used as a treatment against dysentery (Sbah, Di Palma 2016, de Groot, Frissen 2017).

**3.2. FMT in XXI century**

FMT standards are regulated by EUTCD guidelines (The European Union Tissue and Cells Directives). The consensus was elaborated in 2016 at the European conference, and in 2017 a guide with a set of recommendations was published. FMT guide developed by the European Working Group, set standards for the selection of stool donors, the method of material preparation and the route of FMT administration (Cammarota, Ianiro et al. 2017). A group of researchers from Denmark very precisely developed the FMT transplant procedure. It is a good idea to look for
fecal donors among blood donors who already
have some of the diagnostic tests done; such as
collected blood counts or serological blood tests
for HIV, cytomegalovirus (CMV), hepatitis B and
C (HBV, HCV), Epstein-Barr (EBV) and syphilis.
Donors are recruited among people aged 25-50, in
general healthy, without obesity but also without
malnutrition (BMI Body Max Index 18-28 kg/m²).
The excluding factor is permanent drug usage, the
use of antibiotics in the last 6 months, risky be-
haviors (sexual relations with unknown partners,
tattoos, piercing body parts, trips to countries with
low hygiene standards), intestinal diseases and
other chronic diseases, including depression.
In additional, blood tests are performed to evaluate
the functions of specific organs: pancreas (amy-
lase), kidneys (creatinine), liver (ALT, AST, bili-
rubin, INR, albumin) and the level of electrolytes,
CRP, immunoglobulins and glycated hemoglobin.
Besides the DNA of the parasites Strongyloides
tercoloralis and Entamoeba histolytica, Cryptospor-
dium spp. is also sought. Fecal sample are also
analyzed for presence of enteropathogenic bacteria
such as C. difficile (toxinogenic and ribotype 27),
EPEC, Salmonella spp., Shigella spp., C. jejuni,
Y. enterocolitica, multi-drug resistant bacteria and
several viruses (adenovirus, enterovirus, paracho-
Currentl, FMT is mainly used to treat rCDI
and IBD. The FMT may be administrated by naso-
gastric tube, nasoduodenal tube, esophagogudo-
scopy (EGD), colonoscopy, or enema (Khan-
na, Pardi 2012, Rohlke, Stollman 2012, Landy,
Al-Hassi et al. 2011). The volume of material for
FMT may also vary, depending on the route of ad-
ministration. When using a nasogastric tube, 25-
50 ml is given, and 200 to 500 ml when during
colonoscopy. There are also differences regarding
the administration of a fresh or frozen sample, the
time that elapses from donating feces to the prepara-
tion of FMT (6-24h). Researchers also suggest
bowel lavage before FMT and loperamide after co-
lonoscopy to stop diarrhea or probiotics use (Kim,
Gadani et al. 2018, Rohlke, Stollman 2012). In
a study of 35 patients infected with C. difficile and
subjected to the FMT procedure, it was confirmed
that 30 patients (85,7%), achieved improvement
and there was no recurrence of CDI. Patients had
previously undergone conventional treatment with
metronidazole, vancomycin or fidaxomycin, but
this did not work. Among group of 5 patients
infected with hypervirulent strain 027, 60% efficacy
was achieved, suggesting that the C. difficile strain
027 is not easily treated by the FMT (Kim, Gada-
ni et al. 2018). This procedure, however, seems to
be very evaluative and gives hope for the treatment
of other diseases, e.g. it can be used against multi-resistant microorganisms, such as rod-sha-
pered Gram-negative bacteria possessing New Dehi
metabolobacterialactamase (NDM), extended-spectrum
beta-lactamases (ESBL), Klebsiella pneumoniae
carbenapenemase (KPC), OXA 48. Biliński et al.
described the case of treating oncology patient
colonized by multi-resistant bacteria, including K.
pylori NDM (+) and E. coli ESBL (+) with FMT.
The patient with hematologic disease was
undergoing neoplastic therapy in strong neutrope-
nia. There was a high risk of systemic multi-drug
resistant (MDR) infection. Antibiotic therapy did
not work, but after the use of a FMT, MDR strains
were not found in the control cultures (Biliński,

3.3. FMT in oncology

There are indications that bacterial dysbiosis,
and in particular the presence of specific groups
of bacteria may lead to carcinogenesis. The pro-
cesses that promote tumor formation are chron-
ic inflammation and damage to the host’s DNA.
Dysbiosis leads to a decrease in the production of
short-chain fatty acids and activation of inflam-
mation through TLRs. Some bacteria produce
proteins that promote the separation of β-caten-
in from E-cadherin, activating β-catenin signal
pathway involved in carcinogenesis (Chen, Wu
et al. 2018). β-catenin regulates cell proliferation
and differentiation by regulating transcription
factors, controls the adhesion and migration of
cells, while in the complex with E-cadherin, it
builds intercellular connections (Tian, Liu et al.
2011). Intercellular junctions provide the integrity
of tissues, their development and proper matura-
tion. They enable interaction and transmission of
signals between neighboring cells and between
them and the extracellular matrix. Weakening of
cell adhesion may lead to disturbance of cell cy-
uclear control (Kwiatkowski, Godlewski et al. 2009).
Changes in the composition of the intestinal mi-
crobiota may lead to the development of cancer:
colorectal cancer (CRC), hepatocellular carcino-
ma (HCC), pancreatic cancer, breast cancer, and
melanoma. Although the impact of long-term an-
tibiotic therapy on cancerogenesis is debatable.
Helicobacter pylori, Bacteroides fragilis, Strepto-
coccus gallolyticus, pathogenic Escherichia coli,
Fusobacterium nucleatum, are species that have
been proven in cancerogenesis. H. pylori is clas-
sified by the World Health Organization (WHO)
as a class I carcinogen and results in gastric ade-
enocarcinoma and mucos-associated lymphoid
tissue (MALT lymphoma). H. pylori virulence
factors stimulate the signal path leading to tumor
development (Wang, Meng et al. 2014). Chronic
inflammation and oxidative stress, which leads to
damage of the host DNA are main causes of carcinogenesis. Very important is *H. pylori* cytotoxin (CagA) and its other virulence factors such as VacA, urease and NapA2 (Ajagopala, Vashee et al. 2017). The bacterium uses a type IV secretion system to translocate CagA to gastric epithelial cells, activating β-catenin signaling pathway (Müller 2012). *B. fragilis* toxin causes inflammation and DNA damage in host cells, leading to the development of colorectal cancer (CRC). In the CRC tissue, *S. gallolyticus* is also present, additionally activating β-catenin signaling pathways in mice. The pathogenic strains of *E. coli* can produce calmodulin, leading to the development of a tumor. *F. nucleatum* promotes proliferation and the ability to adhere tumor cells to tissue (Chen, Wu et al. 2018, Cammarota, Ianiro et al. 2017).

In CRC patients, changes in the composition of the intestinal microbiota were noted - a decrease in the number of *Lactobacillus* and *Bifidobacterium* genus, and an increased number of the *Staphylococcus*, *Fusobacterium* genus, and the *Peptostreptococcus anaerobius* species. On the basis of experiments carried out on mice, it was found that butyric acid producers such as *Clostridium butyricum* and *Bacillus subtilis* may promote the development of CRC tumor, while the probiotic bacteria *Lactobacillus casei* strain BL23 counteracts. It was also noticed that in germ-free mice after transplantation of the intestinal microbiota from patients with CRC, the tumor develops, whereas after transplantation of feces from wild mice this development is stopped (Chen, Wu et al. 2018).

Oncological patients are exposed to long-term hospitalization, the use of anti-cancer drugs, immunosuppressants and antibiotic therapy. These factors also may predispose to the development of the cancer. The type and malignancy of the tumor may affect predisposition to CDI, but there are no clearly defined correlations between the type of cancer and the risk of development of CDI (Garzotto, Garcia et al. 2015, Abughanimeh, Qasrawi et al. 2018). Researchers estimated the prevalence of CDI in the general hospitalized population at 1-2%, while the incidence was 7-14% in adult oncological patients (Scappaticci, Perissinotti et al. 2017). In patients during chemotherapy, CDI is estimated at 7% (Chung, Kim et al. 2016). The prevalence of CDI in patients undergoing bone marrow transplantation (HSCT) is 9 times higher than in general population and 1.4-fold higher than that of other oncology patients (Chopra, Chandrasekar et al. 2011). CDI also occurs 15 times more often in children with cancer compared to the pediatric population without cancer (Tai, Richardson et al. 2011).

At the present time, FMT seems to be a safe procedure. However, considering that it consists of the administration of biological material from the donor, the question arises whether the method of treatment of recurrent CDI is also safe in oncological patients, especially those with immunosuppression and neutropenia. Hefazi et al. Examined 23 patients with various tumors (hematologic and visceral) using the FMT procedure for the treatment of recurrent CDI. The majority of patients improved, CDI recurrence occurred only in 2 patients, but it was cured with standard therapy (metronidazole, vancomycin and fidaxomycin) without any problems. It turned out that this method is also safe for non-immunocompetent patients with multiple comorbidities (Hefazi, Patnaik et al. 2017).

CDI is one of the main complications in patients undergoing hematopoietic stem cell transplant. Long-term hospitalization, antibiotic therapy, chemotherapy and radiotherapy damaging the intestinal mucosa and microbiota promote the development of CDI. A higher percentage of CDI is observed among allogeneic compared with autogenous transplant recipients. This can be explained by a higher frequency of hospitalization, a greater impairment of the immune system and longer antibiotic therapy. The development of CDI also predisposes to the development of acute graft-versus-host disease (GI GVHD) (Alonso, Treadway et al. 2012).

The FMT procedure for HSCT recipients in the treatment of CDI, including recurrent cases, is possible. This method is effective regardless of the patient’s age and route of administration (gastro-intestinal tube, capsuled feces). There is also evidence that FMT prevents and treats patients with acute graft-versus-host disease GI GVHD, because after administration of FMT in patients’ stool the number of bacteria from the genus of *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium* increases and intestinal microenvironment is restored. Increasing the diversity of microorganisms in the feces of HSCT patients leads to a decrease in side effects after transplantation including CDI development (De Filipp, Hohmann et al. 2019).

4. Discussion

FMT is a procedure involving the administration of a specially prepared stool samples from healthy donor to a recipient who has qualitative and quantitative disturbances in the normal intestinal microbiota (Limketkai, Hendler et al. 2019). Bacteria produce different substances (of which the most important are short-chain fatty acids – SCFAs and secondary bile acids, polyamines and vitamins), which may, affect the development of cancer and the effectiveness of anti-cancer therapies (Zitzovg, Daillère et al. 2017).

The FMT procedure was first used in 1958 in 4 patients suffering from pseudomembranous colitis, when Eisman et al. used the enemas from...
donor feces (Eisman, Silen et al. 1958). Intestinal microbial disorder can lead to dysbiosis. It was noted that critical patients hospitalized in ICU have a disturbed intestinal microbial composition (Lankelma, van Vught et al. 2017). Dysbiosis is also a predisposing factor for the development of many serious diseases, including cancer and CDI. (Chen D. Wu J. et al. 2018, Cammarata, Ianiro 2017). There is a connection between the composition of the intestinal microbial composition and the development of cancer. Microorganisms as H. pylori, F. nucleatum, Epstein-Barr Virus (EBV) and Human Papilloma Virus (HPV) are well known carcinogenesis factors, which may have direct oncogenic effects, by producing toxins, damaging the epithelial barrier, impairing the anticancer immune surveillance, stimulating the production of trophic factors such as growth factors or other proinflammatory cytokines (Zitvogel, Daillère et al. 2017).

In the literature there is a broadly described, close connection between intestinal dysbiosis and the development of colorectal cancer. The composition of the intestinal microbiota of CRC patients differs from the microflora of the control group patients (Helmink, Khan et al. 2019, Sears, Garrett 2014, Gao, Guo et al. 2015). In CRC tumor tissues, the presence of F. nucleatum was found, moreover this bacterium may also contribute to resistance to anti-cancer chemotherapy (Yu, Guo et al. 2017). Experiments in mice have shown that the use of the FMT procedure may reduce colorectal carcinogenesis (Bel, Elkins et al. 2014).

Recently, an increase in C. difficile incidence has been observed. The main factors predisposing to CDI are older age, long-term hospitalization and the use of antibiotics. Important factors contributing to the development of CDI are immunosuppression and achlorhydria (Barlett 2017).

Oncological patients are hospitalized for a long time, undergo immunosuppressive therapy (hemato-oncological patients undergoing bone marrow transplantation) and preventive antibiotic therapy. All of these increases risk of CDI (by 10%) (Alonso, Treadway et al. 2012). Hematological malignant tumors are an independent risk factor that causing the development of CDI (Dubberke, Reske et al. 2007). The use of fecal transplantation in patients with multiple comorbidities seems to be very risky. Stool is a material containing a large number of microorganisms, including pathogenic ones. Therefore, it is very important to keep all safety procedures during the selection of the donor and during the FMT procedure itself. In oncological patients, the FMT procedure also has positive effects in the treatment of CDI, without causing excessive side effects (Abu-Sbeih, Ali et al. 2019). With all precautions taken, the FMT procedure can be used in cancer patients both for CDI treatment and for the eradication of multiresistant bacteria (Biliński, Grzesiowski. et al. 2016).

5. Short conclusion

For most people FMT seems to be a highly controversial procedure, having features of alternative medicine. Since the first application of FMT in 1958, there has been a significant development of medicine. Research methods have developed (including molecular ones, allowing to evaluate the bacterial microbiome), the approach to the concept of bacterial microbiota inhabiting the human body has also changed. While the XX century enraptured with antibiotics, the XXI century, aware of the growing antibiotic resistance among bacteria and the severity of CDI, reduce this admiration. FMT is a procedure that belongs to non-standard therapies. Based on the observations described above, it can be concluded that it is relatively safe, and above all, effective in the treatment of an increasing number of diseases, including comorbidities of oncological patients.

References:


