Fecal microbiota transplantation in the treatment of intestinal steroid-resistant graft-versus-host disease: two case reports and a review of the literature

Monika Maria Biernat¹, Donata Urbaniak-Kujda¹, Jarosław Dybko², Katarzyna Kapelko-Słowik¹, Iwona Prajs¹ and Tomasz Wróbel¹

Abstract
Acute graft-versus-host disease (aGvHD) reduces the efficiency and safety of allogeneic hematopoietic stem cell transplantation (allo-HSCT). In recent years, attempts have been made to transplant fecal microbiota from healthy donors to treat intestinal GvHD. This study presented two cases of patients undergoing allo-HSCT who were later selected for fecal microbiota transplantation (FMT). In the first patient, FMT resulted in the complete resolution of symptoms, whereas therapeutic efficacy was not achieved in the second patient. FMT eliminated drug-resistant pathogens, namely very drug-resistant Enterococcus spp., but not multidrug-resistant Acinetobacter baumannii or Candida spp. Further research is needed, particularly on the safety of FMT in patients with intestinal steroid-resistant GvHD and on the distant impact of transplanted microflora on the outcomes of allo-HSCT. FMT appears promising for the treatment of patients with steroid-resistant GvHD.

¹Department and Clinic of Haematology, Blood Neoplasms, and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland
²Department and Clinic of Internal and Occupational Diseases and Hypertension, Wroclaw Medical University, Wroclaw, Poland

Corresponding author:
Monika Biernat, Department and Clinic of Haematology, Blood Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University, Pasteura Street 4, 50-367, Wroclaw, Poland.
Email: mobiernat@gmail.com
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Introduction
Acute graft-versus-host disease (aGvHD) reduces the efficiency and safety of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The condition is usually severe, and it carries a high mortality rate. GvHD may manifest with skin, intestinal, and liver involvement.1 Intestinal localization is one of the most severe forms of the disease. Disturbance of the mechanisms responsible for homeostasis of the immune system in the intestine and excessive responses of the donor’s cytotoxic lymphocytes play crucial roles in the development of this form of GvHD. In the course of aGvHD, the mucosal barrier in the intestine is disturbed by conditioning treatment, resulting in the release of bacterial lipopolysaccharides and pro-inflammatory cytokines and the subsequent activation of immune response receptors and the cytokine storm.2 In addition, earlier treatment with broad-spectrum antibiotics and colonization with multidrug-resistant (MDR) bacteria before transplantation may also be important in the pathogenesis of aGvHD and may influence its course.3,4 Corticosteroids and immunosuppressive drugs are used routinely as the initial therapies. However, the effectiveness of these treatments is unsatisfactory, and there are no drugs with proven effectiveness against steroid-resistant GvHD. Because of the lack of effective treatment options for intestinal GvHD, it is necessary to identify new methods for preventing and treating this complication after allo-HSCT.

In recent years, the importance of intestinal microflora in maintaining gastrointestinal homeostasis has been increasingly described.5 On the basis of mouse models and research studies in patients with gastrointestinal tract diseases, it has been proven that the quantitative and qualitative composition of the gastrointestinal microflora plays an important role in the pathogenesis of many human diseases, such as inflammatory bowel diseases, metabolic diseases, autoimmune diseases, allergies, and infectious diseases.1,3,6 Understanding the importance of the gastrointestinal microflora in the course of GvHD may contribute to the development of new therapeutic strategies.7,8 In recent years, attempts have been made to transplant intestinal microflora from healthy donors as a treatment for intestinal GvHD. Therefore, this study presented two cases of patients undergoing allo-HSCT who were selected for fecal microbiota transplantation (FMT).

Case 1 presentation
The first case concerned a 25-year-old male patient with acute myeloid leukemia that was diagnosed in September 2016. The bone marrow examination identified 97% of the cells as various myeloid dendritic cells, which immunophenotypically were classified as CD11c+, CD56+, CD123+/-, and CD4-. On the basis of cytogenetic and molecular studies, the patient was diagnosed with 9;11(p22; q23) translocation with MLL and NPM1 (+) rearrangement, i.e., intermediate cytogenetic risk II.
The treatment was based on an induction regimen using the DAC protocol (daunorubicin, cytarabine, cladribine). After the patient achieved complete remission with minimal residual disease, treatment was continued with a consolidation regimen using the high-dose cytarabine (HiDAC) protocol. The patient qualified for allo-HSCT using cells donated by his brother. The Cy-Bu regimen was used as the conditioning regimen, and prophylaxis for GvHD consisted of cyclosporine and methotrexate. In the transplant, the patient received $8.38 \times 10^6$ CD34\(^+\) cells/kg recipient body weight. The evidence for hematological restoration (engraftment) was as follows: granulocytes >500/μL on day 16 after transplantation and platelets >20.0 G/L on day 17 after transplantation. The patient received ciprofloxacin, fluconazole, and acyclovir as routine anti-infective prophylaxis according to the local protocol. No infection or GvHD was observed in the early post-transplantation period. The post-transplant chimerism test result revealed 100% donor T-cell chimerism. On day 34 after transplantation, the patient was readmitted to the Department of Hematology because of diarrhea (10–12 bowel movements/day, approximately 2 L/day) and abdominal pain. Laboratory tests revealed anemia, elevated concentrations of CRP, fibrinogen, and lactate dehydrogenase (LDH), and decreased total protein and serum albumin levels. An infectious cause of diarrhea, including parasitic or viral infection and bloodstream infection, was excluded. The patient, who was afebrile, underwent rectosigmoidoscopy, and an analysis of biopsy samples confirmed the diagnosis of grade II aGvHD. Cytomegalovirus-induced enteritis was excluded. The intestinal form of aGvHD (grade IV) was also detected. Initially, methylprednisolone was administered at a dose of 2 mg/kg/day. A stool culture was performed, revealing the presence of Enterococcus faecalis, Escherichia coli extended-spectrum beta-lactamase-positive (ESBL\(^+\)), Candida albicans, and Klebsiella pneumoniae ESBL\(^+\). Additionally, the patient was diagnosed with neutropenic fever, followed by pneumonia. For treatment, broad-spectrum antibiotics, initially including meropenem and vancomycin, were used, followed by meropenem and linezolid, which were co-administered with anti-fungal agents (voriconazole). Because the patient’s diarrhea did not improve after approximately 3 weeks of treatment, immunosuppressive therapy (infliximab and budesonide, followed by methotrexate and then cyclophosphamide) was intensified. To treat severe abdominal pain, morphine was added to the regimen. Additionally, immunoglobulin was administered. Because of the persistence of diarrhea and progressive cachexia, it was decided to include parenteral nutrition. The presence of BK virus DNA in urine and plasma without dysuria was found in viral tests. After 3 months, because of the ineffectiveness of the therapy, FMT was performed as a rescue therapy with the prior written consent of the patient and the consent of the Bioethical Commission of Wroclaw Medical University. Fecal suspensions used for transplantation were obtained from healthy donors of Caucasian race (age, 20–40 years) as previously described. Briefly, active bacterial, viral, fungal, and parasitic infections were excluded in the donors, especially HAV, HBV, HCV, HIV, CMV, EBV, syphilis, intestinal parasites, Clostridium difficile, and enteropathic flora. Donors were not treated with antibiotics for 3 months before sampling, they were in good overall health, they had normal BMIs, and they followed a regular diet. Each product was derived from 100 g of feces. Fecal suspensions were prepared at the Center for Research and Transplantation of Intestinal Microbiota, Center for Preventive Medicine and...
Rehabilitation in Warsaw according to a previously described protocol and immediately transported to the Wroclaw Transplant Center within 5 hours. FMT was performed as follows. Patients were fasted on the day of transplantation, and no medications were administered. In the morning and 30 minutes before the procedure, pantoprazole was administered at a dose of 40 mg intravenously, and approximately 150 to 250 mL of fecal samples suspended in physiological salt were administered through an intranasal probe previously installed by a physician, after which the probe was removed. An easily digestible diet was applied, and a control examination of feces was performed 7 days after the procedure. The second procedure was performed no sooner than 7 to 10 days after the first procedure. Antibiotics were temporarily stopped prior to each FMT administration.

The patient underwent FMT three times without complications. Fecal culture was performed 7 days after each transplantation (Table 1). A gradual improvement of gastrointestinal symptoms, mainly a decrease in the amount of diarrheal stools, was observed after the second FMT, and the symptoms were completely resolved a couple of days after the third FMT, occurring in the sixth month of hospitalization. The patient was discharged in good general condition.

Case 2 presentation
The second case concerned a man with cerebral palsy who was admitted to the Department of Hematology at an age of 32 years. The patient was diagnosed with osteomyelofibrosis at an age of 31 years and categorized as intermediate risk 1 according to the DIPSS plus scale. The diagnosis of osteomyelofibrosis was made in the regional hematological ward on the basis of trepanobiopsy (presence of fibrosis [collagen fibers], MF1/2 approximately 80%), and the presence of the V617F mutation in the JAK2 gene. On admission, the patient was in a good condition. The morphological examination revealed severe normocytic and normochromic anemia, mild leukopenia according to the World Health Organization classification, and elevated LDH levels. For condition, the patient was treated according to the Cy-Bu protocol. On day 0, stem cells were administered at 7.43 × 10^6 CD34+ cells/kg recipient body weight. The patient was diagnosed with liver GvHD. Methylprednisolone was readministered at a dose of 2 mg/kg/day. Additionally, because of deterioration of his bilirubin level, extracorporeal photopheresis (ECP) was performed. On the fifth day of hospitalization, the patient developed a bloodstream infection caused by MDR Acinetobacter baumannii related to the indwelling catheter, and he died in the intensive care unit of multiorgan failure.
presence of BK virus with viral loads of 1465 copies/mL in plasma and 1.3078 \times 10^7\) copies/ml in urine). Intensive fluid therapy was administered, resulting in the resolution of symptoms and reduced viral loads in urine and plasma. On day 42 after transplantation, diarrhea occurred (up to 15 stools/day, approximately 2.5 L/day). The patient was afebrile, and infectious parameters (e.g., C-reactive protein, procalcitonin) were negative. The patient underwent ultrasonography and CT, which revealed thickening of the intestinal wall in the ileum. The patient was disqualified

Table 1. Results of the analysis of stool specimens from patients.

|                      | Patient 1                              | Patient 2                              |
|----------------------|----------------------------------------|----------------------------------------|
| Pre-transplant       | *Clostridium difficile*-negative       | *Clostridium difficile*-negative       |
|                      | *Candida albicans*                     | *Enterococcus faecium* (S)             |
|                      | *Enterococcus faecalis* (S)            | *Enterococcus faecalis* HLGR           |
| Post-transplant GvHD | *Clostridium difficile*-negative       | *C. difficile*-negative                |
|                      | *Candida albicans* (S)                 | *Enterobacter cloacae ESBL+*           |
|                      | *Klebsiella pneumoniae* (ESBL+)        | *Klebsiella pneumoniae* (S)            |
|                      | *Enterococcus faecalis* (S)            | *Candida albicans* (S)                 |
|                      | *Escherichia coli* ESBL+               | *Candida parapsilosis* (S-voriconazole, R-fluconazole, anidulafungin) |
| Post 1st FMT         | *Escherichia coli* (S)                 | *Enterococcus faecium* HLGR            |
|                      | *Klebsiella pneumoniae* MDR            | *Candida albicans* (S)                 |
|                      | *Candida albicans*                     | *Candida parapsilosis*                 |
|                      |                                         | (S – voriconazole, R – fluconazole, anidulafungin) |
|                      |                                         | *Stenotrophomonas maltophilia*         |
|                      |                                         | (S – trimethoprim/sulfamethoxazole),   |
|                      |                                         | *Klebsiella pneumoniae* (S)            |
| Post 2nd FMT         | *Enterococcus faecium* GRE***          | *Stenotrophomonas maltophilia*         |
|                      | *Klebsiella pneumoniae* PDR****        |                                         |
|                      | *Acinetobacter baumannii* MDR****      |                                         |
|                      | *Candida albicans*                     |                                         |
| Post 3rd FMT         | *Klebsiella pneumoniae* (S)            | *Enterococcus faecium* VRE             |
|                      | *Candida albicans* (S)                 | *Candida albicans*                     |
|                      | *Acinetobacter baumannii* MDR****      |                                         |
| Post 4th FMT         | Not done                               | *Escherichia coli* (S)                 |
|                      |                                         | *Citrobacter freundii* (S)             |

FMT, fecal microbiota transplantation; S, susceptible to all tested antibiotics; R, resistant to at least one of the tested drugs; GvHD, graft-versus-host disease, ESBL+, extended-spectrum beta-lactamase positive; MDR, multidrug-resistant; PDR, pan-drug-resistant; HLGR, high-level aminoglycoside-resistant

E. coli ESBL+ *(S – netilmicin, meropenem, ertapenem, pipercillin/tazobactam, gentamicin, amikacin; R – ciprofloxacin, cefuroxime, ceftazidime, ampicillin/sulbactam, levofloxacin, trimethoprim/sulfamethoxazole), Klebsiella pneumoniae MDR** – all, (S – colistin, imipenem, tigecycline), Enterococcus faecium GRE*** (R – ampicillin, imipenem, vancomycin, teicoplanin, gentamicin, S – linezolid), Klebsiella pneumoniae PDR* (R – all, S – colistin), Enterococcus faecium HLGR (S – vancomycin, teicoplanin, linezolid, R – ampicillin, imipenem, gentamicin), Acinetobacter baumannii MDR***** – all, (S – colistin, tobramycin), Enterobacter cloacae ESBL+ (R – all, S – meropenem, ertapenem, imipenem, colistin).
from endoscopy because of the severe clinical condition and the increased risk of perforation. The diagnosis was the intestinal form of aGvHD (grade IV). Fecal culture revealed positivity for \( E. coli \) ESBL (+), \( Enterococcus faecium \) GRE, \( Candida albicans \), \( C. parapsilosis \), \( K. pneumoniae \), \( Stenotrophomonas maltophilia \), and \( C. difficile \) GDH antigens, whereas \( C. difficile \) toxins A and B were not detected. Corticosteroids (methylprednisolone 2 mg/kg/d IV) were administered for 2 weeks, followed by calcineurin inhibitors and infliximab (anti-TNF-\( \alpha \)) at a dose of 10 mg/kg recipient body weight. Moreover, because of pneumonia and neutropenic fever, empiric antibiotic therapy (ceftazidime and vancomycin followed by imipenem-cilastatin and linezolid) and antifungal drugs (voriconazole) were administered. CMV DNAemia in the early stage of replication was detected on day 51 after transplantation. Intravenous ganciclovir (5 mg/kg every 12 hours) was administered, and after 14 days, the treatment was changed to maintenance therapy. Control CMV quantitative PCR was negative.

Because of the lack of clinical improvement and persistence of diarrhea, the patient underwent FMT as a rescue therapy based on the prior written consent of the patient and the consent of the Bioethical Commission of Wroclaw Medical University. FMT was repeated four times with a minimum interval of 7 days. Fecal culture was performed after each transplantation (Table 1). After the third and fourth rounds of FMT, a temporary reduction in symptoms, i.e., pain and the amount of stool, was observed (approximately 1/day). \( E. coli \) and \( Citrobacter freundii \) were detected in stool samples after administration of the fourth round of FMT without resistance mechanisms. Because of the recurrence of diarrhea within 1 week and the presence of liver dysfunction characterized by elevated liver enzymes above \( 3 \times \) the upper limit of normal and total bilirubin levels exceeding 15 mg/dL, the patient qualified to undergo ECP. No episode of CMV or EBV reactivation was detected. He required parenteral nutrition, blood, platelets, and plasma substitution. Only temporary improvement of the patient’s condition was achieved with the applied treatment. Death occurred on day 128 after transplantation with symptoms of multiorgan failure.

**Discussion**

In recent years, the role of the microbiome and its evolution in patients who underwent allotransplantation have been reported.\(^{10,11}\) It was demonstrated that a conditioning chemotherapy regimen and total-body irradiation lead to changes in the quantitative and qualitative composition of the intestinal flora. Chemotherapeutic agents used in conditioning regimens decrease the counts of species, mainly \( Clostridium \) and \( Bifidobacterium \) spp., and increase those of bacteria in the genus \( Enterococcus \).\(^{1,12}\) The reduction in the diversity of the intestinal microflora appears to be an independent factor influencing mortality in the course of GvHD.\(^{13–15}\) Patients undergoing allo-HSCT develop long-term dysbiosis, which plays an essential role in GvHD pathogenesis.\(^{11,12,16}\) Of importance, dysbiosis leads to the prevalence of one type of bacterium or fungus and acute inflammation.\(^{14}\) In addition, it was demonstrated that the early use of broad-spectrum antibiotics, especially carbapenems and piperacillin/tazobactam, in the treatment of infections after allo-HSCT increased mortality associated with intestinal GvHD.\(^{17–19}\) The use of FMT to restore the normal microbiome can be an attractive option in the treatment of intestinal GvHD. To date, there have been few reports on this subject. FMT was used in auto-HSCT and allo-HSCT recipients to treat \( C. difficile \) infection.\(^{20}\) Bilinski et al.\(^9\)
used FMT in eight allo-HSCT recipients who developed infections caused by MDR bacteria. These researchers observed that these bacteria were eradicated in 75% of the patients within 1 month. The use of FMT in patients with steroid-resistant intestinal GvHD was discussed in single-institution studies and in one pilot study. The authors observed an almost complete resolution of symptoms and few adverse effects of the treatment in most patients. In our center, FMT was applied in two patients as described previously. In both cases, patients underwent all-HSCT, and post-transplantation chimerism analysis revealed 100% donor T-cell chimerism. Both patients were diagnosed with severe intestinal aGvHD and treated with standard therapy, which was ineffective. CMV reactivation was identified in one patient, whereas BK virus-associated viremia was detected in both patients. The frequency of BK virus infection in the post-transplant period is estimated to be approximately 7% to 70%, whereas CMV reactivation develops in more than 60% of seropositive recipients. CMV reactivation may exacerbate GvHD and increase transplant-related mortality. In the first patient, three rounds of FMT resulted in complete symptom resolution, and the death of the patient was not related to the FMT procedure. However, according to the culture analysis, the patient developed a catheter-related bloodstream infection by *A. baumannii* with the same phenotype as the strain previously isolated from the patient’s feces. It appears that the patient was permanently colonized by this *Acinetobacter* strain, but the occurrence of a new infection cannot be excluded. The aspect of the eradication of MDR microorganisms, especially of the genus *Enterococcus* VRE and MDR rods of the *Enterobacteriaceae* family, using FMT has resulted in conflicting results. Moreover, the impact of FMT administration on the eradication of *Acinetobacter* and other non-fermenting rods is unknown. In the second patient, the therapeutic effect was mediocre, but FMT eliminated drug-resistant pathogens and temporarily improved the patient’s symptoms. No adverse events were observed after FMT in either patient. However, both patients ultimately died. As demonstrated by the culture results, previously detected bacteria such as *K. pneumoniae* and MDR *Enterococcus* species were not isolated after the second and third rounds of FMT, confirming the reports of other authors on the elimination of MDR bacteria by this procedure. However, it should be noted that FMT carries some risks for adverse infectious events, which was demonstrated by DeFilipp et al. The authors described two patients who developed bacteremia caused by ESBL-producing *E. coli* after FMT from the same stool donor. The effect of FMT on the mycobiome is unknown, and few relevant reports have been published. The observations in our patients indicate the constant presence of *Candida* fungi in patients’ feces independent of FMT. Invasive mycoses are common and extremely serious complications in the post-transplant period, and prophylactic fluconazole reduces the frequency of these infections as well as the severity of GvHD. Van der Velden et al. found that dysfunction of dectin-1, an innate receptor for fungi, was associated with increased colonization by *Candida* species in human recipients and the aggravation of aGvHD. Moreover, studies using mouse models reported that the injection of fungal mannan and heat-killed *C. albicans* exacerbated GvHD in the lungs. The effect of colonization with non-fermenting rods and *Candida* fungi on the course of GvHD requires further research. Another aspect is the use of ECP in patients with steroid-refractory GvHD and its interaction with FMT. We used...
ECP in our patients, mainly because of the worsening of the condition of both patients. The utility of ECP in the treatment of GvHD has not yet been established. Although the exact mechanism of action is unknown, reports on small groups of patients are promising, especially concerning the treatment of cutaneous and intestinal GvHD.\textsuperscript{40–42}

The present report had some limitations. We presented only two cases, both of which were fatal despite the use of all available treatment methods, illustrating the difficulty in treating GvHD. We were unable to perform metagenomic analyses, which would be useful for analyzing material from donors and tracking changes in the microflora after subsequent transplantations. Recent studies revealed that metagenomic analysis targeting the 16S rRNA of intestinal bacterial flora permit the analysis of the molecular mechanisms by which the microbiota affect the clinical outcomes of patients and assessments of changes in the composition and fluctuations of microbiota after transplantation, which cannot be achieved using standard culture methods.\textsuperscript{43} In particular, classical culture methods do not allow analyses of the composition of particular bacterial genera because many of these microorganisms do not grow on standard culture media. Furthermore, reports have found that using next-generation sequencing analysis that it is possible to demonstrate the prognostic significance of each intestinal bacterial genus in patients who underwent allo-HSCT. It has been demonstrated that increased abundance of the genus \textit{Enterococcus} as detected using 16S rRNA sequencing, but not using stool culture techniques, was associated with poor survival in patients who underwent -allo-HSCT.\textsuperscript{44} Little is known regarding the relationship between FMT and CMV reactivation and the impact of FMT on the intestinal mycobiome.

**Conclusion**

In the future, FMT may emerge as a supportive treatment for GvHD, but additional research is needed to assess its safety and efficacy in patients with intestinal steroid-resistant GvHD. Future research should also be conduct to improve donor selection and remotely monitor recipients. It appears that monitoring of the occurrence of the main groups of microorganisms responsible for maintaining intestinal homeostasis in patients undergoing HSCT during the post-transplantation period may be one of the elements that will clarify the relationship between the intestinal microbiome and infections in the post-transplantation period, especially in the course of GvHD.

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**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

**Ethics and consent to publish**

The study protocol was approved by Biotechnological Commission of Wroclaw Medical University. The patients participating in the study provided verbal informed consent for publication.

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**ORCID iD**

Monika Maria Biernat  
https://orcid.org/0000-0003-3161-3398
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