Fecal microbiota transplantation before or after allogeneic hematopoietic transplantation in patients with hematologic malignancies carrying multidrug-resistance bacteria

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Supplemental materials

Patient screening and preventive measures

In our center weekly screening for MDRB is performed in order to identify asymptomatic carriers with high risk of spreading MDRB to other patients. Screening modalities consist of weekly rectal swab. After MDRB identification, patients colonized with vancomycin-resistant (VRE) or carbapenemase-producing enterobacteriaceae (CPE) are cohorted and cared for by dedicated staff, as these two classes of bacteria are classified as emerging XDR (eXDR), i.e. bacteria that present an emerging infection control challenge widely in France. Of note, when those patients are candidates to rehabilitation centers before being discharged at home, they cannot be easily admitted to other healthcare facilities that often do not dispose of dedicated staff [7].

Furthermore, in contact patients, defined as those patients having shared paramedical and/or medical healthcare workers with one or more patients colonized with VRE or CPE, cohorting is also warranted, with initial caring by another dedicated staff until three negative screening tests.

It is worth underlying that opportunistic saprophytic bacteria, such as CP-*Pseudomonas aeruginosa,* have not been considered as eXDR in national guidelines. However, it has been already reported that patients experiencing systemic infections from CP-*Pseudomonas aeruginosa* have a high risk of death[5], and in our Center three consecutive patients (data not published) died during the aplastic phase of allo-HSCT due to bloodstream fatal infections from CP-*Pseudomonas aeruginosa*.

For these reasons, patients colonized with CP- *Pseudomonas aeruginosa* were considered at high risk of fatal complications and, despite not needing isolation and caring by dedicated staff, FMT was proposed to patients experiencing systemic infections or in those colonized in order to limit systemic infections.

A minimal platelet count of 20 x 10^9/L was preferred in order to proceed to the FMT and use of platelet transfusion to reach that threshold before FMT was allowed.

Microbiological testing

For each patient, one rectal swab specimen was plated onto selective media: a screening medium designed to detect ESBL-producing enterobacteriaceae, ChromID ESBL (bioMérieux) and another designed to detect CP-bacteria, ChromID CARBA SMART (bioMérieux). A second rectal swab was used in an enrichment procedure, consisting of an overnight culture at 37°C in a specific broth before plating onto a screening medium designed to detect VRE, ChromID VRE (bioMérieux). All plates were incubated overnight at 37°C. Colonies growing on these selective media were identified at the species level by MALDI-TOF spectrometry. The production of ESBL was determined by an antibiogram and visualization of the characteristic “champagne cork” synergy between amoxicillin-clavulanate and third-generation cephalosporins disks. Carbapenemase production was determined by molecular analysis using the GeneXpert technology (Cepheid) and the Xpert Carba-R kit version 2 (detecting the most prevalent carbapenemases in France, OXA-48 and OXA-48-like enzymes, as well as NDM enzymes). Furthermore, VRE were also identified using the GeneXpert technology (Cepheid) and the Xpert VanA/VanB kit.

In patients achieving decolonization, rectal swabs and/or stool cultures were initially performed weekly and then at each follow-up visit. In patients considered as having achieved total and persistent decolonization, last follow-up for decolonization was considered as the date of the last available negative microbiological culture.

Patients and donors characteristics

The current study was approved by the Ethic Committee. Each patient signed an informed consent mentioning all potential risks of the procedure as described in the paper. According to French regulations in such cases, each patient case was extensively discussed and approved as part of an "RCP" (Réunion de Concertation Pluridisciplinaire") which is a sort of large multidisciplinary meeting aimed to discuss difficult cases and approve unusual therapeutic procedures. The minutes and

decisions of the RCP are recorded in writing, including the names of the participants and their feedback. Patients are informed about this discussion prior to signing the informed consent.

Large spectrum antibiotics were discontinued in the recipients 48-72 hours prior to the procedure and, when possible, use of antibiotics was avoided during at least 72 hours after the procedure.

Stools were preferentially obtained from healthy related or unrelated donors. Of note, related donors not necessarily coincided with allo-HSCT donors. According to regulatory recommendations, potential donors were selected after a previous questionnaire. Donor age was preferentially between 18 and 65 years. Excluded were people who had presented digestive disorders (i.e. diarrhea) within the 3 months prior to donation or having a chronic disease and/or chronic treatments, cases with antibiotic intake within 3 months before the donation, people having been living in the tropics during the three months prior to donation or having been hospitalized abroad for more than 24 hours in the 12 months prior to donation. History of typhoid fever was also considered as exclusion criteria. In people fulfilling inclusion criteria, a complete biological and microbiological assessment was then performed including: serology for *Treponema pallidum*, human immunodeficiency virus, Human T-Lymphotropic Virus, Hepatitis A, B and C, cytomegalovirus, Epstein-Barr virus, amebiasis, *Strongyloides strecoralis* ; stool examination for standard culture, *Clostridium difficile*, multi-resistant bacteria, norovirus, Cryposporidium, parasites. If the biological and microbiological panel was negative, a minimum of 50 g of stools were collected.

Fecal material, prepared as described below, was delivered either by enema or via nasogastric tube. A bowel preparation was performed the day before the FMT by administration of 4 liters of polyethylene glycol (PEG) based solution. For nasogastric administration, patients had to fast for at least 12 hours before transplantation and they received proton pump inhibitors the day before and the morning of the FMT. In the case of enema administration, patients were asked to retain the product for at least 2-3 hours.

Product preparation

Transplants were prepared in the Saint Antoine Hospital pharmacy. In case of freezing, the stool preparation is usually performed in two steps. In the first, preparation and freezing, the stools are manipulated in an extractor hood dedicated to this activity, in the 6 hours following emission. A total of 50-100g stools are weighted and mixed with a sterile cryopreservative saline solution (300mL glycerol+ saline solution 0.9% 10/90 V/V) using sterile blender, containers and medical devices (syringes, filters). The suspension is filtrated through sterile gauze compresses mounted in a funnel to remove solid residues, before freezing at -80°C. If in screening tests an exclusion criterion is fulfilled, the suspension is destroyed. The second step of the preparation procedure starts the day before FMT, when the frozen microbiota solution is placed in a refrigerator (between 4 and 8°C) for an overnight thawing. The thawed suspension is then transferred either to an enema bag (lower gastro intestinal tract delivery) to which 200mL of sterile saline solution are added, or to 50-mL syringes (colonoscopy or nasoduodenal delivery) as ready to be used. . On the other hand, when FMT is performed with fresh stools, fecal materials need to be prepared the day of FMT within the 6 hours following stools emission. In this case stool preparation is performed in a single step, without freezing.

Safety testing of the fecal product was done according to French recommendations[1].

1) Sokol H, Galperine T, Kapel N, et al. Groupe Français de Transplantation Fecale (GFTF). Transplantation de microbiote fecal dans le cadre des infections a Clostridium difficile recidivantes : recommandations pour la pratique clinique courante. Hepato Gastro 2015; 22: 278-290.