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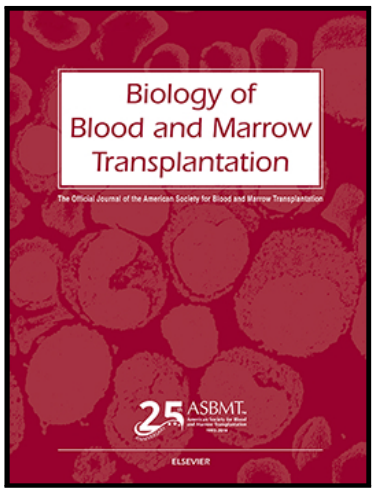


## Dynamics of the Gut Microbiota in Children Receiving Selective or Total Gut Decontamination Treatment During Hematopoietic Stem Cell Transplantation

Vincent Bekker , Romy D. Zwittink , Cornelis W. Knetsch , Ingrid M.J.G. Sanders , Dagmar Berghuis , Peter J. Heidt , Jaak M.J.J. Vossen , Willem M. de Vos , Clara Belzer , Robbert G.M. Bredius , Peter J. van 't Hof , Arjan C. Lankester , Ed J. Kuijper

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**Highlights**

- Selective and total gut decontamination differentially affect the gut microbiota
- Selective decontamination results in a microbiota with high *Bacteroides* abundance
- Stringent bacterial eradication results in a variable residual microbiota
- Probiotics use after HSCT does not aid colonization by the administered bacteria

# DYNAMICS OF THE GUT MICROBIOTA IN CHILDREN RECEIVING SELECTIVE OR TOTAL GUT DECONTAMINATION TREATMENT DURING HEMATOPOIETIC STEM CELL TRANSPLANTATION

Vincent Bekker[1,#], Romy D. Zwittink[2,3,#], Cornelis W. Knetsch[2], Ingrid M.J.G. Sanders[2], Dagmar Berghuis[1], Peter J. Heidt[1], Jaak M.J.J. Vossen[1], Willem M. de Vos[4,5], Clara Belzer[4], Robbert G.M. Bredius[1], Peter J. van 't Hof[6], Arjan C. Lankester[1,*] and Ed J. Kuijper[2,3,*]

[1]: Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands

[2]: Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands

[3]: Center for Microbiome Analyses and Therapeutics, Leiden University Medical Center, Leiden, the Netherlands

[4]: Laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands

[5]: RPU Human Microbiome, University of Helsinki, Helsinki, Finland

[6]: Sequencing Analysis Support Core, Leiden University Medical Center, Leiden, the Netherlands

[#]: Both first authors contributed equally

*: Both last authors contributed equally

**Short title:** The gut microbiota of pediatric HSCT patients

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**Address of Correspondence:**

Romy D. Zwittink

Medical Microbiology, Leiden University Medical Center

Albinusdreef 2, 2333 ZA, Leiden, The Netherlands

r.d.zwittink@lumc.nl

+31715266797

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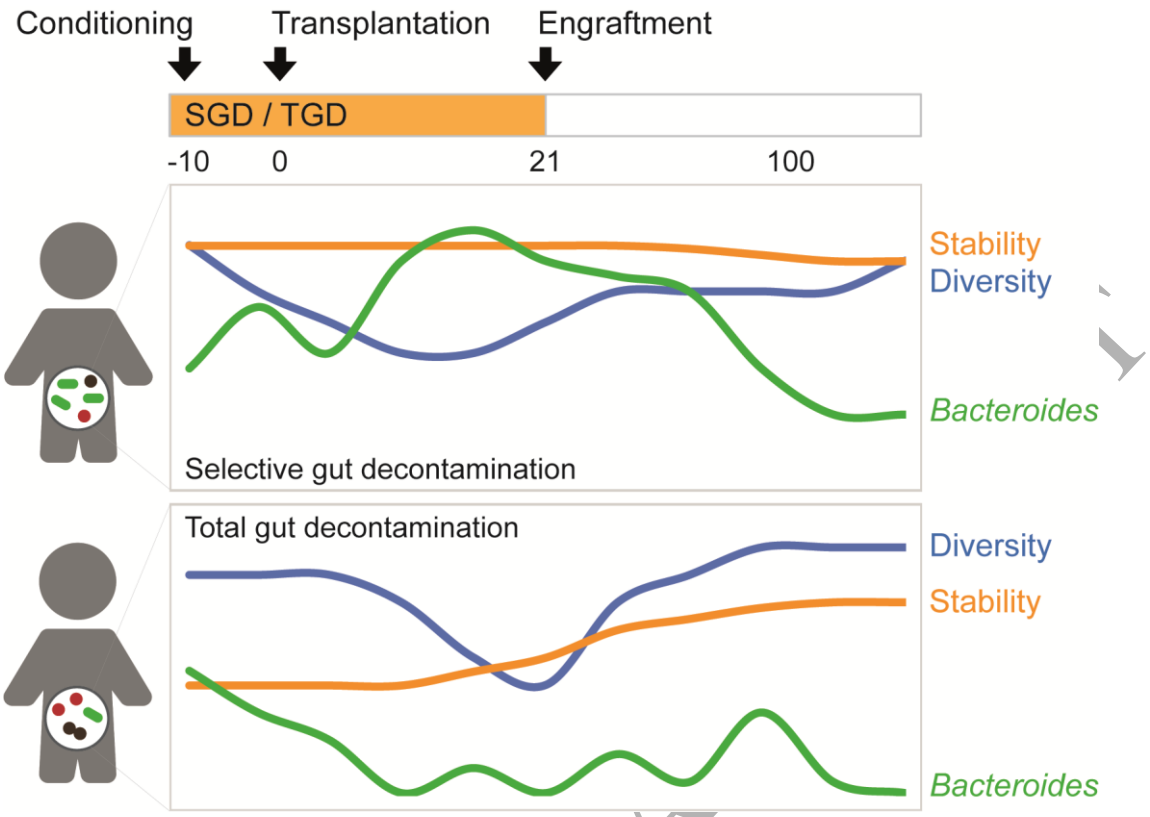
None.

**Graphical abstract**




**Abstract**

Bloodstream infections and Graft-versus-Host disease (GvHD) are common complications after hematopoietic stem cell transplantation (HSCT) procedures, associated with the gut microbiota which acts as a reservoir for opportunistic pathogens. Selective gut decontamination (SGD) and total gut decontamination (TGD) during HSCT have been associated with a decreased risk of developing these complications after transplantation. However, since studies have shown conflicting results, the use of these treatments remain subject of debate. In addition, their impact on the gut microbiota is not well studied. The aim of this study was to elucidate the dynamics of the microbiota during and after TGD and to compare these to the dynamics of SGD. In this prospective, observational, single-center study, fecal samples were longitudinally collected from nineteen children eligible for allogenic HSCT (TGD n=12, SGD n=7), weekly during hospital admission and monthly after

discharge. In addition, fecal samples were collected from three family stem cell donors. Fecal microbiota structure of patients and donors was determined by 16S rRNA gene amplicon sequencing. Microbiota richness and diversity markedly decreased during SGD and TGD and gradually increased after cessation of decontamination treatment. During SGD, gut microbiota composition was relatively stable and dominated by *Bacteroides,* while it showed high inter- and intra-individual variation and low *Bacteroides* abundance during TGD. In some children, TGD allowed the genera *Enterococcus* and *Streptococcus* to thrive during treatment. A gut microbiota dominated by *Bacteroides* was associated with increased predicted activity of several metabolic processes. Comparing the microbiota of recipients and their donors indicated that receiving a stem cell transplant did not alter the patient's microbiota to become more similar to that of its donor. Overall, our findings indicate that SGD and TGD affect gut microbiota structure in a treatment-specific manner. Whether these treatments affect clinical outcomes via interference with the gut microbiota needs to be further elucidated.

**Keywords**

Gut decontamination, Microbiota, Hematopoietic stem cell transplantation, Graft-versus-Host disease, Pediatrics

**Introduction**

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment option for patients with various life-threatening diseases such as high-risk hematological malignancies, acquired or inborn bone marrow failure syndromes, severe immune deficiencies, and hemoglobinopathies. Graft-versus-Host disease (GvHD) is a severe and frequent complication of HSCT, characterized by severe organ damage due the initiation of an immune response of donated tissue (the graft) towards host tissue. The exact pathophysiology of GvHD is not known. Antigen-presenting cells, cytokines and T-lymphocytes play a central role in the pathogenesis of GvHD[1]. GvHD is considered to be initiated by a cascade of inflammation caused by tissue damage and translocation of intestinal microbial components[1].

Mouse experiments revealed that antibiotic exposure before HSCT was a risk factor for the development of GvHD[2]. The bacterial community remained heterogeneous in mice without GvHD, whereas it became severely restricted in mice with GvHD[2]. Also, in adult HSCT patients with GvHD, a loss of diversity of the gut microbiota over time occurred in contrast to patients without GvHD[2]. Patients that later on developed GvHD had a higher Bray-Curtis dissimilarity index, interpreted as greater microbial 'chaos', early after HSCT, before the onset of clinical symptoms[2]. These findings indicate a role for the gut microbiota in GvHD pathogenesis.

Complete suppression of the intestinal microbiota by the use of non-absorbable antibiotics has been shown to prevent the initiation of the inflammatory cascade mediated by translocation of microbial compounds and the subsequent occurrence of acute GvHD[3-5]. Based on this concept, our pediatric HSCT program routinely applies total gut decontamination (TGD), starting at least one week before stem cell infusion, in all T cell replete HSCT patients. If the risk for GvHD is considered low, as in the case of an identical twin as donor or an HLA-identical donor with serotherapy, the patients receive selective gut

decontamination (SGD) as infection prevention during the neutropenic phase. SGD aims at selectively eliminating and suppressing Gram-negative bacterial pathogens and yeasts from the intestinal microbiota. In contrast to studies supporting gut decontamination treatment in the course of HSCT, several studies have associated gut decontamination with an increased risk for GvHD[6,7], rendering their application subject of debate. Many hospitals practice gut decontamination according to their (inter)national guidelines with own adjustments[8]. Gut decontamination procedures therefore vary between centers, which may in part explain the discrepancies regarding GvHD outcomes.

It is incredibly relevant to improve our understanding of the effect of gut decontamination treatments on HSCT outcomes, and to elucidate underlying mechanisms (such as treatment-specific modulation of the gut microbiota). Although some microbiota analyses have been performed in patients undergoing SGD[2,9], it is currently unresolved to what extent the intestinal microbiota is eliminated by TGD. In addition, limited studies focused on microbiota composition during HSCT in children[10,11]. The aim of the current observational, single-center study was to get insight into the gut microbiota structure during and after SGD and TGD in the course of pediatric allogenic HSCT.

## Materials and Methods

### *Subjects*

For this prospective, observational, single-center study, all children (<18y) eligible for an allogeneic HSCT at the Leiden University Medical Center between January and December 2015 were asked to participate in the MiCaDO (Microbioom en Calprotectin in Darm Onderzoek) study. Indications for allogeneic stem cell transplantation were either a malignancy (ALL and AML: n=7), primary immunodeficiency (n=3), myelodysplastic syndrome or Fanconi anemia (n=5) or hemoglobinopathy (sickle cell disease and ß-thalassemia: n=4). Conditioning regimens were chosen based on international protocols with local adaptations, decided upon after group discussion for each patient individually. No *ex-vivo* T cell depletion was applied. Twelve patients received TGD from 10 days before transplantation, until engraftment or 21 days post-transplantation, whichever occurred latest. TGD consists of oral piperacillin/tazobactam and oral amphotericin B[4]. The efficacy of TGD was tested by weekly stool culture. In case persistent growth of aerobic Gram-negative bacteria or yeasts was observed, additional non-absorbable antimicrobials were added based on susceptibility testing. Recolonization of the intestine after TGD was aided by the oral administration of Symbiolact® (SymbioPharm, Germany), which contains *Lactobacillus acidophilus, Lactobacillus paracasei* and *Bifidobacterium lactis*. In case of a haplo-identical peripheral T cell depleted stem cell graft with low T cell counts, HLA-identical donor with serotherapy or a HLA-identical cord blood graft, the chance of the occurrence of GvHD is considered low. In these instances, no TGD was given. Seven patients received SGD instead, consisting of oral polymyxin/neomycin and oral amphotericin B following a similar schedule as TGD, but without the oral administration of Symbiolact®.

***Ethical considerations***

This study was conducted according to the principles of the Declaration of Helsinki, last amended October 2013 (www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and was approved by the medical ethical committee of the Leiden University Medical Center, accession number P14.266. All children and/or their parents provided written informed consent for stool collection and analysis.

***Fecal sample collection***

Fecal samples were collected 10 days before admission, then weekly during admission for transplantation, and monthly thereafter up to 6 months after transplantation. This timeline corresponds to sampling during the 4 weeks of decontamination treatment, up till 6 months thereafter. A total of 120 samples were collected for analysis (Figure S1). In addition, a fecal sample was collected from three family stem cell donors. Fecal samples were stored at -80°C within 24 hours after collection.

***16S rRNA gene sequencing***

DNA was extracted from feces using the Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (ZymoResearch, CA, USA) according to manufacturer instructions. Quality control, library preparation and sequencing were performed by GenomeScan B.V. (Leiden, The Netherlands) using the NEXTflex™ 16S V4 Amplicon-Seq Kit (BiooScientific, TX, USA) and the Illumina 2500 system (rapid mode, paired-end, 250bp). Raw sequencing data is available in the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under study accession PRJEB28845.

Sequencing data was analyzed using the QIIME software package v1.9.1 applying Uclust and the Greengenes database for OTU picking and taxonomic classification[12,13]. The obtained OTU table was filtered for OTUs with a number of sequences less than 0.005% of the total number of sequences[14]. To account for variation between samples' total number of reads, rarefaction to 116076 reads per sample was applied. Microbiota composition profiles for each sample are shown in figure S2. Microbiota richness and diversity were determined by the Chao1 and PD whole tree indexes, respectively. To determine the variation in bacterial community profiles within children over time, weighted and unweighted UniFrac distances were determined. This indicates the dissimilarity in microbiota composition between samples over time from each individual and thereby illustrates overall microbiota stability. To study (dis)similarities in microbiota composition and relate changes in microbiota composition to clinical data, principal component analysis (PCA) and redundancy analysis (RDA) were performed using the Canoco multivariate statistics software v5[15]. For RDA analysis, variables included in the original model were type of decontamination, time since start of decontamination, age, gender, and meropenem, ciprofloxacin, vancomycin, ceftazidime and azithromycin use. These factors were considered significant when the false discovery rate corrected p-value was below 0.05. Microbiota profiles were tested for similarity between donors, between recipients, between donors and their corresponding recipients and between donors and non-corresponding recipients via Spearman correlation. For this purpose, recipients' fecal samples collected around 100 days after transplantation were used to allow for microbiota development and stabilization. Functionality of the microbiota was predicted using PICRUSt 1.0.0.[16], which was presented as relative KEGG orthology profiles. These profiles were imported in Canoco multivariate statistics software v5 for principal component analysis to visualize functional (dis)similarities between samples[15].

## Results

A total of 19 patients and 3 donors were included in this study. Baseline characteristics, underlying disease and type of gut decontamination are outlined in Table 1. Of the 19 patients, 12 received TGD, consisting of oral piperacillin/tazobactam and oral amphotericin B, whereas the other seven received SGD with oral polymyxin/neomycin and oral amphotericin B. Ten children completed 6 months of follow-up, 2 patients died during follow-up, 7 developed bloodstream infection (BSI), and 4 developed GvHD (Table 1).

### *Selective and total gut decontamination decrease microbiota richness and diversity*

To determine the effect of SGD and TGD on gut microbiota structure, 16S rRNA gene amplicon sequencing was performed on longitudinally collected fecal samples from each patient giving insight in the temporal dynamics of bacterial microbiota composition, richness and diversity. During the first two weeks after start of SGD and TGD, microbiota richness (chao1) and diversity (phylogenetic diversity) showed high inter-individual variation (Figure 1a,b). When richness and diversity where higher at start, a temporal decrease could be observed until the point was reached, after four weeks of decontamination, where all children's microbiota was low in richness and diversity (Figure 1a,b). Richness and diversity gradually increased after discontinuation of SGD and TGD, its pace and pattern being individual-specific, which is partly illustrated by the large distinction between the boxplot's mean, minimum and maximum values (Figure 1c,d).

### *SGD, but not TGD, allows a stable gut microbiota dominated by Bacteroides*

To determine (dis)similarities in microbiota composition and relate these to clinical data, ordination analyses were performed. This revealed that type of decontamination treatment was the main driver of variation in microbiota composition between all samples (9.1%),

followed by time since start of decontamination treatment (5.3%) and meropenem use (4.1%) (Figure 2a, Table S1). The difference in microbiota composition between patients receiving TGD or SGD was mainly associated with the abundance of the *Bacteroides* genus (Figure 2a,b). The microbiota of patients receiving SGD was characterized by high abundance of *Bacteroides*, which showed a trend of increasing abundance during decontamination treatment, while decreasing after treatment cessation (Figure 3a, Figure S3a). In contrast, a marked decrease in *Bacteroides* abundance was observed during TGD (Figure 3a). In addition, the gut microbiota of some patients receiving TGD almost solely consisted of *Enterococcus* or *Streptococcus* during treatment, which was not seen in patients receiving SGD (Figure 3b,c, Figure S3b,c). After cessation of TGD, the recolonization attempt via oral administration of Symbiolact®, which contains *Lactobacillus acidophilus, Lactobacillus paracasei* and *Bifidobacterium lactis*, did not affect the abundance of lactobacilli and bifidobacteria.

Microbiota composition was more stable in patients receiving SGD compared to TGD, as indicated by lower within patient UniFrac distances, showing that microbiota composition was less dissimilar between samples over time in patients receiving SGD than in patients receiving TGD ($p=0.046$, Figure 4a). Apart from the high abundance of *Bacteroides*, a stable, but individual-specific, microbiota composition was observed during and after SGD (Figure S2a). However, in one child (subject A), microbiota composition varied greatly over time and was generally dominated by one specific bacterial taxa being either *Bacteroides*, *Staphylococcus* or *Enterobacteriaceae*, most certainly due to the occurrence of several infections and therefore extensive exposure to various broad-spectrum antibiotics. The microbiota of patients receiving TGD was not characterized by a specific stable profile, but showed high intra- and inter-individual variation (Figure S2b). This instability was most

prominent during decontamination treatment, and became less apparent after cessation of decontamination treatment and during follow-up (Figure 4b).

***Receiving HSCT does not alter the recipient's microbiota to become more similar to that of its donor.***

Fecal microbiota composition was determined in three stem cell donors around time of transplantation. Fecal microbiota composition varied among the three donors (Figure S2c). One donor was dominated by *Prevotella* (45%), the second donor was dominated by *Prevotella* and *Lactococcus* (21% and 38% respectively), while the third donor's microbiota composition was more evenly distributed with high abundance of various taxa including *Bifidobacterium*, *Faecalibacterium* and *Lachnospiraceae* (16%, 15% and 15% respectively). *Bacteroides* was a prominent member of the bacterial community in all three donors, covering 9%, 10% and 14% respectively. Despite varying microbiota composition between stem cell donors, community richness and diversity were similar and in line with adult microbiota characteristics (Figure 1a,b).

Given the intimate interplay between the immune system and the gut microbiota, we wondered if transplantation of a donor immune system, through HSCT, would result in a more donor-like microbiota in recipients. Therefore, Spearman correlations between donor and recipient microbiota composition profiles were determined. Correlations between microbiota composition of 1) donors as a group, 2) recipients as a group, 3) donors and its corresponding recipients, and 4) donors and its non-corresponding recipients were 0.78, 0.66, 0.59 and 0.59, respectively. This indicates that receiving a stem cell transplant did not alter the patient's microbiota to become more similar to that of its donor. Instead, a situation is created in which HSCT recipient's microbiota is variable between patients, but more similar

to one another than to donors, reflecting the individual-specific consequences of HSCT procedures on microbiota composition.

***Prediction of microbiota's function***

To get insight in what the decontamination treatment-associated differences in microbiota composition could mean for functioning of the microbiota, we predicted the microbiotas' metagenome, and thereby its potential functional traits, using 16S rRNA-based taxonomy. This revealed that a gut microbiota dominated by *Bacteroides*, as observed during SGD, was associated with increased activity in several metabolic processes, including energy metabolism and glycan biosynthesis and metabolism (Figure S4). Microbiota composition of patients receiving TGD was associated with increased processes involved in membrane transport, transcription, signal transduction and xenobiotics biodegradation and metabolism (Figure S4).

***Graft-versus-Host Disease***

Four patients in this cohort developed GvHD grade 1 or more, of which two patients received SGD (subjects A and C) and two received TGD (subjects N and Q) (Table 2). Although our study set-up does not allow to conclusively link microbiota to GvHD, due to low GvHD prevalence and lack of samples at the time of GvHD onset, we present the chronology of gut microbiota composition and GvHD onset (Figure S5). Subject A and C both followed the 'typical' SGD dynamics before onset of GvHD, with *Bacteroides* being a dominant member of the community (Figure S5). Microbiota composition of subjects N and Q are conflicting, with a predominance of *Enterococcus* species in subject N, and great bacterial diversity in subject Q (Figure S5). Overall, characteristics of the bacterial community as observed in

patients developing GvHD, were also observed in patients who did not develop this severe complication (Figure S2).

**Discussion**

The gut microbiome has been implicated in various health outcomes, including immune recovery after HSCT and onset of infections and GvHD[17]. It is therefore important to understand the effect of gut decontamination treatments on gut microbiota structure. Here, we studied the dynamics of the gut microbiota in an unique cohort of children receiving selective or total gut decontamination as part of HSCT procedures.

In this cohort, microbiota richness and diversity decreased considerably while receiving TGD or SGD, and tended to increase at an individual-specific pace after cessation of decontamination treatment. Although SGD and TGD affected microbial community richness and diversity in a similar manner, they differently affected microbiota composition. During TGD, *Bacteroides* markedly decreased, most certainly driven by oral administration of piperacillin/tazobactam[18,19]. High abundance of *Bacteroides*, as observed during SGD, has been suggested beneficial in times of gut microbiota disturbance, due to their capacity to drive microbiota reconstruction via interspecies interaction through the degradation of polysaccharides[10,20,21]. In our study, functional prediction indeed revealed that a gut microbiota dominated by *Bacteroides* is associated with increased processes involved in energy and glycan metabolism. Alterations in the microbiotas' functional capacities may be particularly relevant in light of HSCT outcomes, e.g. immune recovery and onset of infections and GvHD[17,22,23]. A higher *Bacteroides* abundance pre-transplantation, has been associated with increased propionate levels and decreased risk of acute GvHD in children[10]. In light of this, low *Bacteroidetes* abundance has been used as incentive for autologous fecal microbiota transplantation in patients who have undergone allo-HSCT[24].

In addition to the differentially abundant *Bacteroides*, the genera *Enterococcus* and *Streptococcus* thrived in some children during TGD, which was not observed in any subject receiving SGD. A marked increase in enterococci, streptococci and members of the *Enterobacteriaceae* family post-transplantation has been previously reported[10,11,25]. Outgrowth and predominance of a selected number of bacterial genera might possess a health risk to the patients, as acute GvHD has been associated with bloodstream infections caused by enteric bacteria, particularly by enterococci[25-27]. However, absolute quantification of enterococci would be required to determine whether these bacteria actually outgrew. Microbiota composition was highly variable between patients and within patients over time. Such instability may be a consequence of low bacterial load as a result of the TGD regimen[28]. Administration of Symbiolact® after TGD, upon engraftment, did not aid colonization of *Lactobacillus* and *Bifidobacterium* species. It, however, remains unclear whether Symbiolact® is beneficial by other means, like quicker recovery of bacterial diversity overall or improved clinical outcomes.

Considering the gut microbiota in the course of HSCT, most studies so far focused on microbial predictors or modifiers of GvHD, most commonly in adult populations[2,10,11,25,29-31]. Overall, these studies revealed that profound disturbances of gut microbiota composition and diversity, as a result of HSCT and associated regimens (e.g. gut decontamination), are associated with acute GvHD. Although specific organisms have been suggested as protective (e.g. *Blautia*, *Faecalibacterium* and *Ruminococcus*), or harmful (e.g. *Enterococcus*, *Streptococcus*, *Escherichia* and *Enterobacter*), their exact contribution regarding GvHD needs to be further elucidated.

Using conventional culturing techniques, our group previously showed that successful TGD is associated with a reduced risk of GvHD in children[4,5]. Through the application of next generation sequencing, however, we herein show that bacterial signatures remain, being of

highly variable composition and with a remarkable decrease of anaerobic *Bacteroides*. The elimination of beneficial bacteria, and its potential consequences for clinical outcomes, should be considered when applying the TGD regimen in the course of HSCT. The low incidence of GvHD in this cohort, in combination with the lack of samples around time of GvHD diagnosis, prevented to study the link between gut decontamination regimen, microbiota composition and GvHD. In this cohort, 37% of patients developed bloodstream infection. Interestingly, this occurred in a higher percentage of patients receiving SGD (57%) than TGD (25%).

Taking into account the gut microbiota of a small number of stem cell donors, we observed that despite the intimate interplay between the immune system and the gut microbiota, a stem cell transplant did not result in the recipient's microbiota becoming more similar to that of its donor. So far, research regarding the microbiota of stem cell transplant donors is limited. High bacterial diversity in transplant donors has been associated with decreased acute GvHD risk[32], but a study using murine models reported no association between the donor microbiota and GvHD severity.

This is, to our knowledge, the first report on gut microbiota dynamics in children receiving two different gut decontamination regimens as infection and GvHD prophylaxis during HSCT. Despite the prospective set-up and longitudinal sampling, the relatively small subject size, lack of pre-decontamination samples and diversity in underlying diseases and other clinical characteristics makes further investigation warranted. In addition, microbiota composition analysis via 16S-rRNA gene amplicon sequencing, as herein, does not provide species-level information nor insight in actual microbiota functioning, which could improve the understanding of the host-microbiota relationship. Nevertheless, our findings give a good indication of the differential effect of SGD and TGD on the gut microbiota during pediatric HSCT. Whether these gut decontamination treatments affect clinical outcomes via the

interference with the gut microbiota still needs to be elucidated. In addition, further research should focus on clinical implication and possibilities to stimulate microbiota recovery after HSCT.

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## Tables

Table 1. **Subject characteristics.**

| Decon | Subject | Age | Gender | Indication | Donor type | SC source | SC donor | BSI episodes* | Causative organism** | Systemic Antimicrobials | GvHD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **SGD***** | A | 16 | Male | Leukemia | MUD 10/10 | PBSC | - | 7 | *Staphylococcus epidermidis* (5), *Klebsiella pneumoniae* (4), *Klebsiella ornitholytica* (1), *Enterococcus faecalis* (1), *Candida orthopsilosis* (1) | Van, Caz, Vcz | GIT/Skin |
| **SGD** | B | 16 | Male | Leukemia | HLA-identical | Bone marrow | Donor 2 | 1 | *Staphylococcus epidermidis* (1) | Vcz | - |
| **SGD** | C | 17 | Male | Benign hematology | HLA-identical | Bone marrow | - | - | - | Van, Caz | Skin |
| **SGD** | D | 8 | Male | Benign hematology | HLA-identical | Bone marrow | - | - | - | Amx, Van, Caz | - |
| **SGD** | E | 8 | Female | Benign hematology | Haploidentical | PBSC | - | 2 | *Streptococcus mitis* (1), *Staphylococcus aureus* (1), *Klebsiella pneumoniae* (1), *Acinetobacter baumannii* (1) | Van, Caz | - |
| **SGD** | F | 10 | Male | Benign hematology | HLA-identical | Bone marrow | Donor 3 | 1 | *Actinomyces oris* (1) | Van, Caz | - |
| **SGD** | G | 11 | Male | MDS | HLA-identical | Bone marrow | Donor 1 | - | - | Van, Caz | - |
| **TGD** | H | 3 | Female | Leukemia | MUD 10/10 | Bone marrow | - | 1 | *Lachnoanaerobaculum orale* (1) | Van, Caz, Vcz | - |
| **TGD** | I | 14 | Female | Leukemia | MUD 10/10 | Bone marrow | - | - | - | Van, Caz, Vcz | - |
| **TGD** | J | 7 | Female | MDS | MUD 10/10 | Bone marrow | - | - | - | Van, Caz | - |
| **TGD** | K | 15 | Female | MDS | MUD 9/10 | Bone marrow | - | - | - | Amx, Van, Caz | - |
| **TGD** | L | 15 | Female | MDS | HLA-identical | Bone marrow | - | - | - | - | - |
| **TGD** | M | 1 | Female | PI | MUD 6/10 | Cord blood | - | 1 | *Bacillus simplex* (1) | Van, Vcz | - |
| **TGD** | N | 13 | Female | Leukemia | MUD | Bone marrow | - | - | - | Van, Caz, Vcz | Skin |
| **TGD** | O | 11 | Male | PI | HLA-identical | Bone marrow | - | - | - | Van, Caz | - |
| **TGD** | P | 1 | Female | PI | MUD 9/10 | Bone marrow | - | - | - | Amx, Van, Caz | - |
| **TGD** | Q | 13 | Male | Benign hematology | MUD 10/10 | Bone marrow | - | 3 | *Moraxella* (2), *Microbacterium paraoxydans* (2), *Streptococcus mitis* (1), *Staphylococcus epidermidis* (1) | Amx, Van, Caz | Skin |
| **TGD** | R | 1 | Male | Leukemia | MUD 10/10 | Bone marrow | - | - | - | Amx, Van, Caz | - |
| **TGD** | S | 12 | Female | MDS | MUD 10/10 | Bone marrow | - | - | - | Van, Caz | - |

* Episode is defined as a two weeks period. ** Number between brackets indicates the amount of sepsis episodes the organism was identified in. *** Patient A received SGD instead of TGD due to the presence of multidrug-resistant organisms in the gut. ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, Amx: Amoxicillin, BSI: Bloodstream infection, Caz: Ceftazidime, Decon: gut decontamination, GvHD: graft-versus-host disease, HLA: Human leukocyte antigen, MDS: Myelodysplastic syndrome, MUD: Matched unrelated donor, PBSC: peripheral blood stem cell, PI: Primary immunodeficiency, SC: stem cell, Van: Vancomycin, Vcz: Voriconazole.

Table 2. **Graft-versus-Host Disease characteristics.**

| **Subject** | **Decontamination** | **Onset** (days since start decontamination) | **Onset** (days since HSCT) | **Organ** | **Grade** |
|---|---|---|---|---|---|
| A | SGD | 65 | 54 | GIT/Skin | 3 |
| C | SGD | 26 | 16 | Skin | 1 |
| N | TGD | 57 | 47 | Skin | 2 |
| Q | TGD | 49 | 39 | Skin | 2 |

GIT: gastrointestinal tract, HSCT: hematopoietic stem cell transplantation.

**Figure legends**

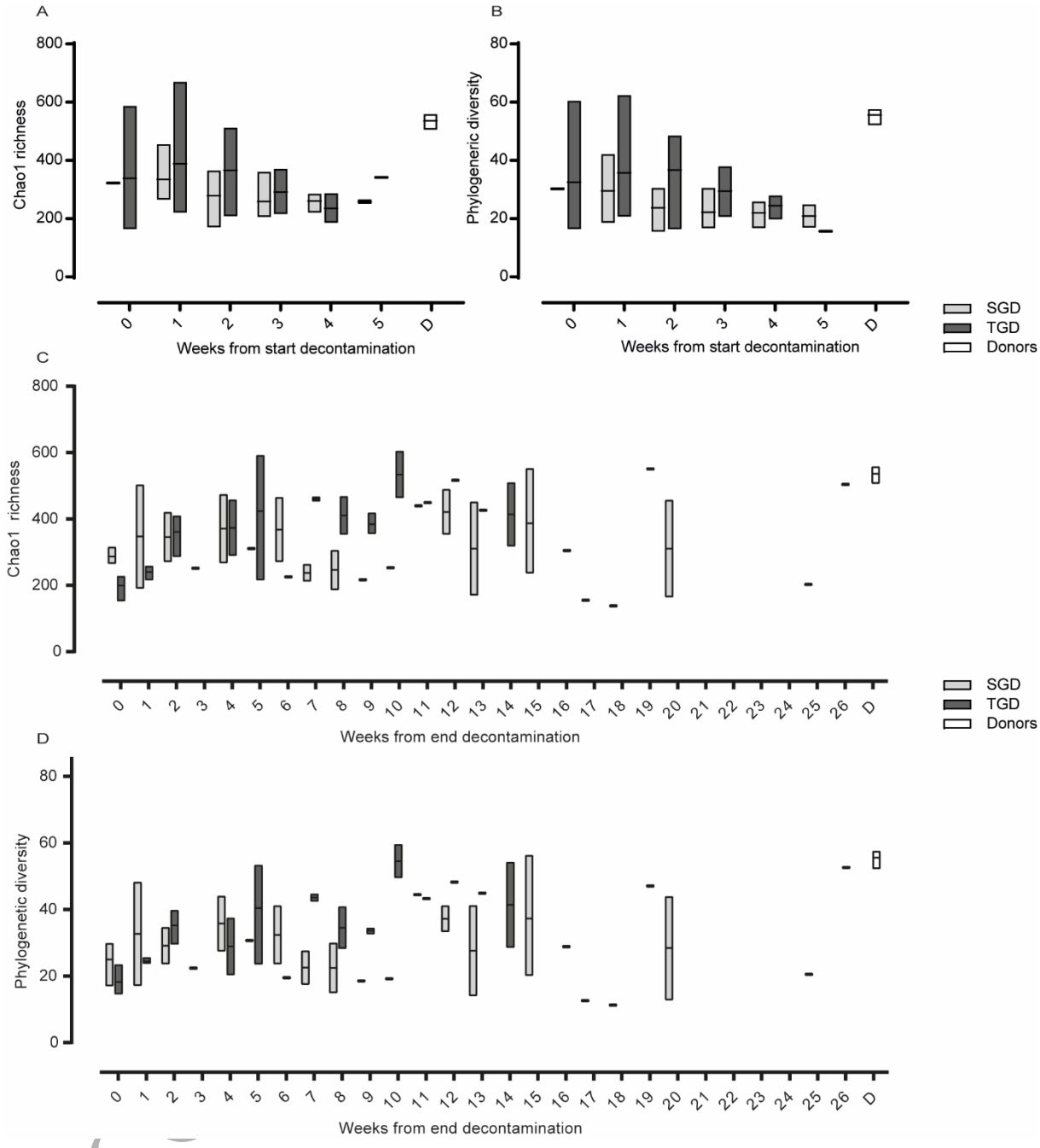


Figure 1. **Chao1 richness and phylogenetic diversity of the bacterial community during (A, B) and after (C, D) decontamination treatment.** Boxes show minimum, maximum and mean values. D: Stem cell donors.

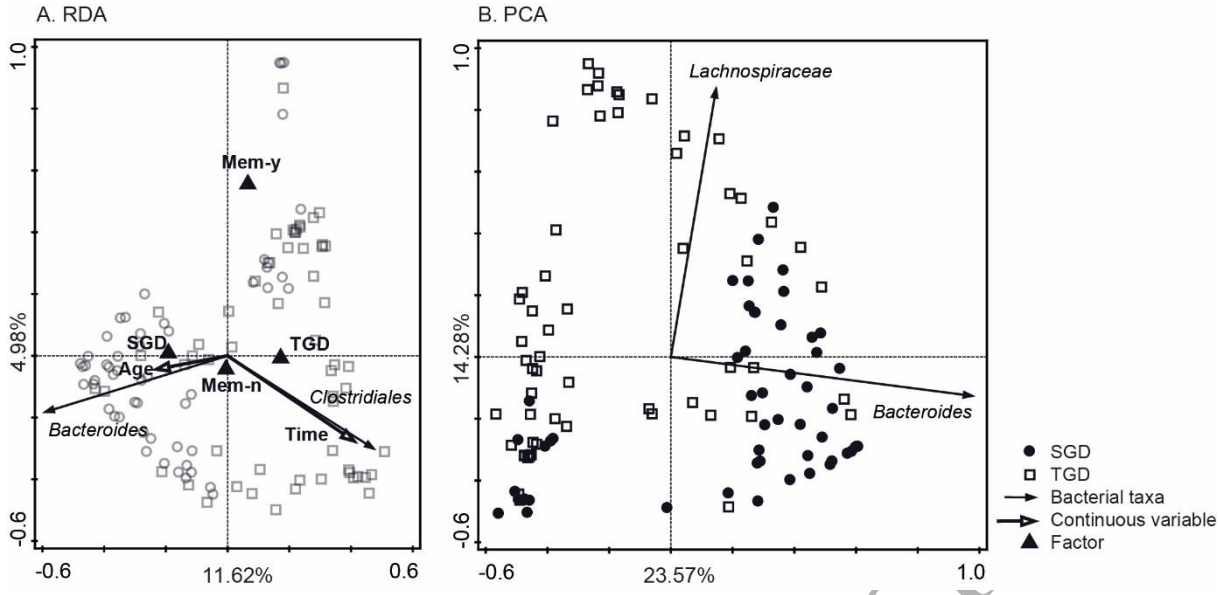


Figure 2. **Redundancy analysis (A) and principal component analysis (B) of the gut microbiota composition profiles.** Per sample taxonomic profiles at genus level were used to generate these plots. Clinical factors significantly explaining microbiota variation are shown. The percentage of variation explained by the principal coordinates is indicated on the axis.

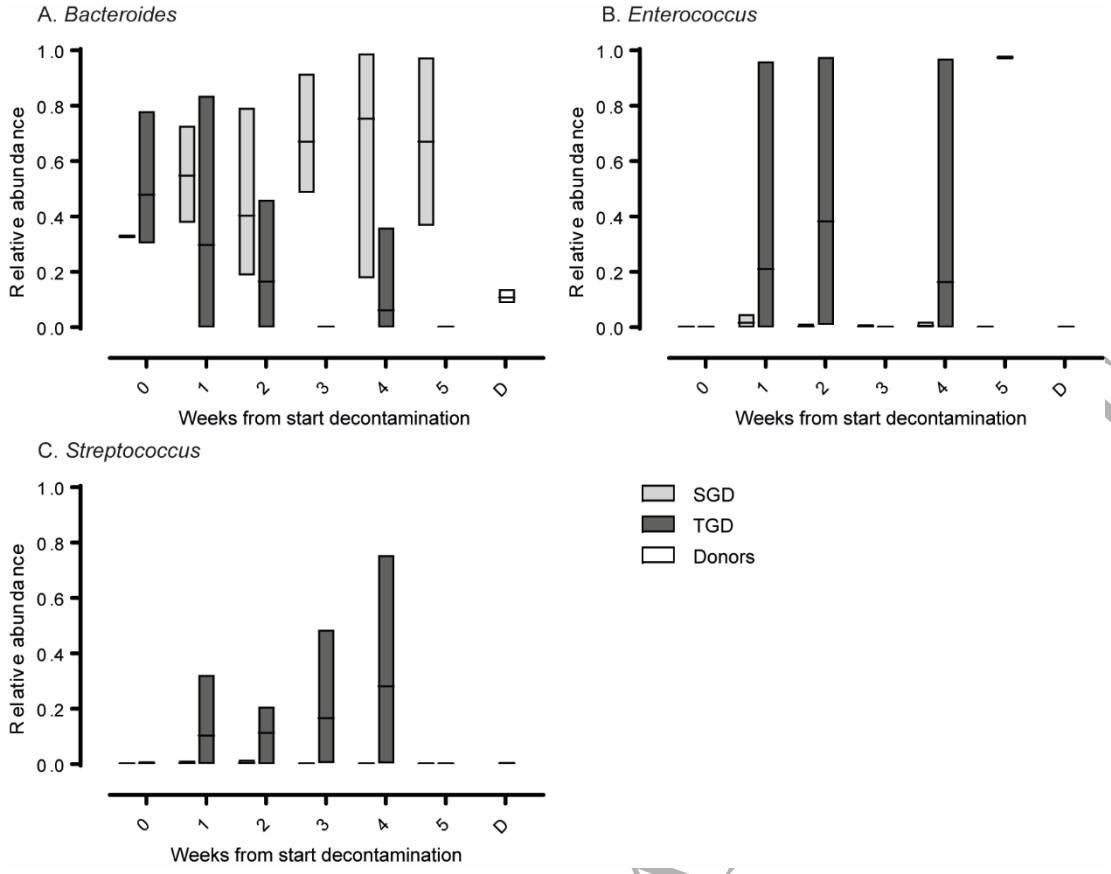


Figure 3. **Temporal dynamics of specific bacterial taxa during decontamination treatment.** Boxes show minimum, maximum and mean relative abundance. D: Stem cell donors.

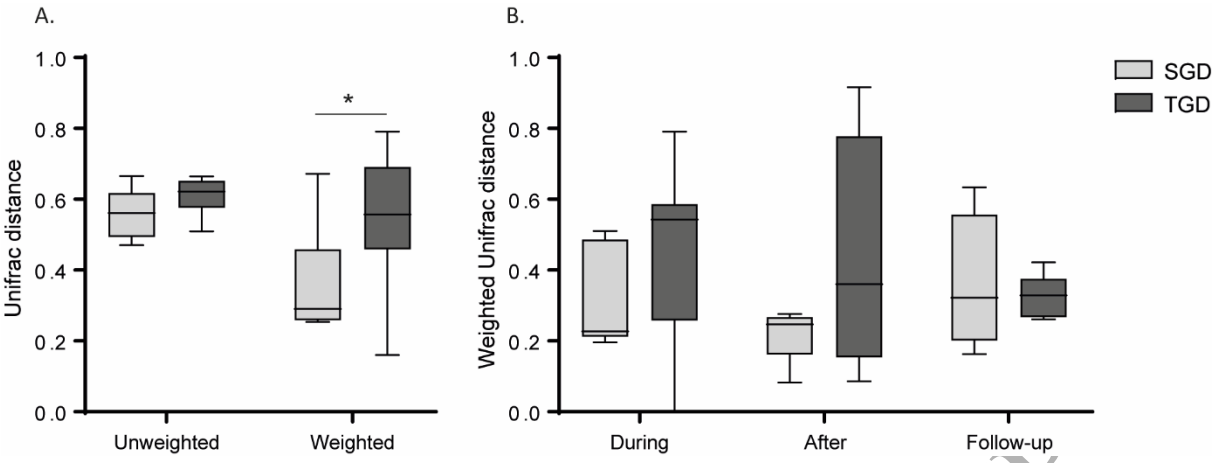


Figure 4. **UniFrac distances within children receiving selective or total gut decontamination treatment.** A. Weighted and unweighted UniFrac distance within children based on all available samples. Boxplots show the median, 25th and 75th percentiles, and minimal and maximal values. The asterisk indicates a significant difference ($p < 0.05$) as determined via the Kruskal Wallis test with Monte Carlo permutation (10.000x). B. Weighted UniFrac distance within children during decontamination treatment (during), during the four weeks after cessation of decontamination treatment (after) and during the months of follow-up (follow-up). Boxplots show the median, 25th and 75th percentiles, and minimal and maximal values. Statistical analysis was not performed since UniFrac distances could not be determined for all subjects at each time period, resulting in insufficient amount of data points for statistical testing.