의학박사 학위논문

소아 조혈모세포이식에서 급성 이식편대숙주병의 발생과 공여자 및 수여자의 위장관 미생물총과의 연관성

The association between acute graft versus host disease and gut microbiome in pediatric allogeneic hematopoietic stem cell transplantation

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서 유 리

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2020년　2월

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2020년 2월

Abstract

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Title: The association between acute graft versus host disease and gut microbiome in pediatric allogeneic hematopoietic stem cell transplantation.

Purpose: Acute GvHD (aGvHD) is an immunological disorder developing by a process in which donor-derived T cells recognize host healthy tissue as non-self after hematopoietic stem cell transplantation (HSCT). Gut microbiome dysbiosis has known to be able to trigger several inflammatory disorders, including aGvHD. However, the association between aGvHD and gut microbiota of the pediatric HSCT recipients were rarely reported.

Methods: We prospectively enrolled pediatric recipients aged younger than or equals 19 years old who underwent allogeneic HSCT at Asan Medical Center Children s Hospital, between September 2018 and Febraury 2019. We collected 3 pairs of fecal samples from both the recipient (R0) and the relevant donor (D0) just before HSCT, and then from the recipient (R1) at 1 month following HSCT. Microbial analysis of fecal samples was performed using 16S rDNA gene sequencing. Operational taxonomic unit-based bacterial diversity was estimated by calculating Chao1 index. The Bray-Curtis dissimilarity was used to compare the diversity among each sample. Demographic data and clinical findings of recipients were abstracted from electrical medical records. Acute GvHD was diagnosed clinically and graded by the International Bone Marrow Transplant Registry criteria.

Results: Total 10 pediatric allogeneic HSCT recipients were enrolled. The median values of Chao1 index in R0 and R1 were 168.9821 (range,

55.8333–289.1539) and 111.5938 (range, 11.5–246.9231), respectively. Average alpha diversity of R1 tended to show a lower diversity by 15% compared to R0. The median value of Chao1 index of D0 was 193.9624 (range, 93.1539–322.0222). Three recipients developed aGvHD within 100 days following HSCT. Two had skin aGvHD of stage 3 and stage 2, respectively. One patient developed gastrointestinal and skin aGvHD of grade 3. The median Chao1 index in R0, R1, and D who developed aGvHD was lower than those in R0, R1, and D who did not develop aGvHD, although not statistically significant. R1 in aGvHD group showed a higher abundance of *Gerbera hybrid cultivar* genus (p = 0.0345) and a lower abdundance of *Erysipelatoclostridium* genus (p = 0.045) compared to those in group without aGVHD. Donors whose relevant recipients experienced aGvHD showed higher abundance of [Eubacterium] *eligens group* genus (p = 0.040). However, we could not find the statistically significant difference in composition of R0 which was associated with aGVHD.

Conclusions: Our results indicated that the gut microbiome of pediatric recipients after HSCT and donor's gut microbiome showed difference between pediatric recipients who developed aGvHD and who did not develop aGvHD. Identifying the gut microbiome of recipients and donors could help predict the occurrence of aGvHD.

Keywords: Acute graft–versus–host disease; Allogeneic hematopoietic stem cell transplantation; Gut microbiota; 16S rDNA gene sequencing; Pediatric patients

차 례

표 및 그림 차례

Introduction

The human gut microbiome, which accounts for more than 90% of all human microbiome, share a mutualistic relationship with human beings.[1-2] The human gut microbiome is becoming recognized as a significant factor in the maintenance of human health and disease.[3-5] It plays an important role in limiting the access of enteropathogens to the gastrointestinal tract and is essential for the normal function of the host local and systemic immunity. The gut microbiome exerts a central role in gut microbiome-host immunological cross talk via a direct interaction or its final metabolites, being strategic to the maintenance of our immune homeostasis. The composition of the gut microbiome is affected by a variety of factors, including host genetic features, diet, medications, infection, chronic disease, and environmental exposures.[6-7] Gut microbiome dysbiosis, which means a significant disruption in the composition and function of the gut microbiome, can trigger several inflammatory disorders, such as acute graft versus host

disease (aGvHD).

Acute GvHD is an immune disorder caused by a process in which donor-derived T cells recognize the recipient tissue as non-self after hematopoietic stem cell transplantation (HSCT). It affects gastrointestinal tract, liver and skin, and one of main complications following HSCT, determining success of HSCT.[8] Several studies have documented that aGvHD is associated with detectable shifts in the composition of the gut microbiome in both murine and human recipients of HSCT. Gut microbiome dysbiosis can be driven by HSCT itself and all related clinical practice including conditioning regimen, antibiotic exposure, and diet.[9-10] In human HSCT recipients, inflammatory reactions of the intestine and aGvHD were associated with the following changes after HSCT; a decrease of bacterial diversity,[11-12] lower abundances of specific commensal bacteria,[13] and increase in the proportion of *Enterococci* and decrease in the commensal bacteria.[9] Although most of the recent human studies were focused on the

association between aGvHD and recipient's gut microbiome after HSCT, the studies revealing the impact of pre-transplant microbiome on development of aGvHD are relatively few. Doki et al., reported that adult patients who developed aGvHD showed a significantly higher abundance of phylum *Firmicutes* and a lower tendency for *Bacteroidetes* before HSCT compared to those without aGvHD.[14] Recent studies by Biagi et al. showed children who experienced acute gastrointestinal GvHD had a lower diversity, lower *Blautia* content, higher abundance in *Fusobacterium* in gut microbiome before HSCT.[15] In addition, children who did not experienced aGvHD had higher abundances of propionate-producing *Bacteroidetes*.[10] The impact of gut microbiome of the stem cell donor on aGvHD as well as gut microbiome of recipient is also receiving attention. Different microbiome of the stem cell donor may promote different compositions of donor immune cells, consequently impacting alloreactivity and aGvHD of recipients.[16-18] However, study of pediatric HSCT recipients are rarely reported.

Therefore, we aimed to elucidate associations between gut microbiome of recipients and aGvHD and to determine the impact of gut microbiota of donor on the occurrence of aGvHD of the recipients in pediatric HSCT.

Materials and methods

1. Study populations

We prospectively enrolled pediatric patients aged ≤ 19 years old who underwent allogeneic HSCT at Asan medical center between September 2018 and February 2019. The following cases were excluded from this study: 1) a refusal of written informed consent, 2) death within one month after HSCT, 3) engraftment failure within one month, 4) relapse of underlying disease within one month after HSCT, and 5) HSCT from unrelated donor.

Age at time of transplantation, gender, the underlying disease leading to HSCT, types of donor (i.e., matched sibling or haploidentical), conditioning regimen (i.e., myeloablation vs. non-myeloablation), engraftment day of

WBC after HSCT, use of GvHD prophylaxis, use of antibiotics for a period between one month before HSCT and one month after HSCT (if used, the type of antibiotics), aGvHD within 100-day follow-up post HSCT, relapse, HSCT related morbidity including CMV reactivation, and death were abstracted from electronic medical records. Antibiotic use at fecal sampling was defined as the use of antibiotics for more than a week on the day of fecal sampling. CMV reactivation was defined as the CMV PCR titer being above 2.69 log copies per 1 mL of whole blood (489 copies/mL) even once. The Institutional Review Board at Asan Medical Center approved this study (IRB No. 2018-0867).

2. Diagnosis of acute GvHD

Acute GvHD was diagnosed clinically by pediatric oncologist, and graded per the International Bone Marrow Transplant Registry criteria (Table 1).[19]

Table 1. International Bone Marrow Transplant Registry criteria

GvHD staging			
Stage	**Skin**	**Liver (total bilirubin)**	**GI tract (diarrhea output/day)**
0	No GvHD rash	<2 mg/dL	Adult: <500 mL/d *Child: <10 mL/kg/d
1	Maculopapular rash < 25% body surface area	2−3 mg/dL	Adult: 500−999 mL/d Child: 10−19.9 mL/kg/d − or − persistent nausea, vomiting, or anorexia with a positive upper GI biopsy
2	Maculopapular rash 25−50% BSA	3.1−6 mg/dL	Adult: 1000−1500 mL/d Child: 20−30 mL/kg/d
3	Maculopapular rash > 50% BSA	6.1−15 mg/dL	Adult: >1500 mL/d Child: >30 mL/kg/d
4	Generalized erythroderma (> 50% BSA) plus bullous formation or desquamation > 5% BSA	>15 mg/dL	Severe abdominal pain with or without ileus, or grossly bloody diarrhea
Overall Clinical Grade : Grade 0: No GvHD of any organ Grade 1: Stage 1−2 skin and no liver OR GI tract involvement Grade 2: Stage 3 skin and/or stage 1 liver and/or stage 1 GI tract Grade 3: Stage 0−3 skin with stage 2−3 liver and/or stage 2−3 GI tract Grade 4: Stage 4 skin, liver, and/or GI tract			

* Use adult values for patients ≥ 50kg

GI = gastrointestinal; GvHD = graft versus host disease

3. Sample preparation

Feces from HSCT recipients were collected twice; pre-HSCT (between one month before HSCT and infusion day), and post-HSCT (one month following HSCT). Stool samples from the relevant donors were collected once; before collection of their stem cells. For each HSCT, three pairs of stool samples were collected as follows; fresh stool was scooped with a spoon attached to the tube lid, and one spoon of stool was put into a collection tube with no additives. It was temporarily stored at at room temperature as short as possible (maximum < 72hr), and then kept in a −70℃ deep freezer until the DNA was extracted.

4. DNA extraction

The stool sample is filtered through a cell strainer after being diluted in 10mL of PBS for 24 hours. To separate the bacteria from stool, bacteria in stool samples are isolated using differential centrifugation at 10,000 x g for 10 min at 4℃. After centrifugation, the pellet is comprised of bacteria. To extract the DNA out of the bacteria membrane from stool, bacteria are boiled for 40 min under 100℃. To eliminate remaining floating particles and waste, supernatant is collected after 30 min at 13,000 rpm of centrifugation under 4℃. DNA is extracted by using a DNA isolation kit (PowerSoil DNA Isolation Kit, MO BIO, USA); The standard protocol is followed as the kit guide. The DNA from bacteria and EVs in each sample is quantified by using QIAxpert system (QIAGEN, Germany).

5. Bacterial metagenomic analysis using DNA

Bacterial genomic DNA was amplified with 16S_V3_F (5′ – TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG –3′) and 16S_V4_R (5′ – GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC –3′) primers, which are specific for V3–V4 hypervariable regions of 16S rDNA gene. The libraries were prepared using PCR products according to MiSeq System guide (Illumina, USA) and quantified using a QIAxpert (QIAGEN, Germany). Each amplicon is then quantified, set equimolar ratio, pooled, and sequenced on a MiSeq (Illumina, USA) according to the manufacturer's recommendations.

6. Analysis of the microbiome composition

Paired–end reads that matched the adapter sequences were trimmed by cutadapt version 1.1.6.[20] The resulting FASTQ files containing paired–end reads were merged with CASPER and then quality filtered with Phred (Q) score based criteria described by Bokulich.[21–22] Any reads were shorter than 350 bp and longer than 550 bp after merging, were also discarded. To identify the chimeric sequences, a reference–based chimera detection step was conducted with VSEARCH against the SILVA gold database.[23–24] Next, the sequence reads were clustered into Operational Taxonomic Units (OTUs) using VSEARCH with de novo clustering algorithm under a threshold of 97% sequence similarity. The representative sequences of the

OTUs were finally classified using SILVA 128 database with UCLUST (parallel_assign_taxonomy_uclust.py script on QIIME version 1.9.1) under default parameters.[25] The Chao Indices, an estimator of richness of taxa per individual, were estimated to measure the diversity of each sample. The Bray-Curtis dissimilarity was used to compare the diversity among each sample.

7. Statistical Analysis

To avoid potential bias caused by differing sequencing depths, samples with >3500 reads were rarefied to a depth of 3500 reads for subsequent analysis. Significant differences between recipients who developed aGvHD and did not develop aGvHD were assessed by Mann-Whitney U test or Fisher's exact test. A p-value < 0.05 was considered statistically significant. The alpha diversity of microbial composition was measured using the Chao1 index and rarified to compare species richness. Principal component analysis (PCA) of Bray-Curtis distances was performed to determine differential clustering between recipients who developed aGvHD and did not develop aGvHD. Comparison of alpha -diversity and beta diverstiy among groups was carried out by Wilcoxon signed-rank test. All statistical analyses were performed using R version 3.4.1.

Results

1. Patients' characteristics

Between September 2018 and February 2019, a total of 10 pediatric HSCT recipients with a median age of 10.8 years (range, 13 months – 19 years) were enrolled (Table 2). Demographic and clinical characteristics of the recipients are summarized in Table 2. Underlying disease leading to HSCT were malignancies including hematologic malignancy or solid tumor (n=8) and hematologic disorders (n=2). Stem cell donors were matched or one allele mismatched siblings (n=5), and haploidentical family donor (n=5). All recipients who underwent HSCT from sibling donor received GvHD prophylaxis with cyclosporin and/or mycophenolate mofetil. In addition, one of the haploidentical HSCT recipients received donor used mycophenolate mofetil for GvHD prophylaxis. Seven recipients received non-myeloablative conditioning.

All recipients used per oral antibiotics including trimethoprim/sulfamethoxazole (TMP/SMX) or ciprofloxacin for gut decontamination from the start of conditioning regimen to day −2. Six recipients with neutropenia before HSCT received systemic antibiotics for prophylaxis during the conditioning regimen.

Table 2. Baseline characteristics of the HSCT recipients

No.	Sex/Age	Underlying disease	Donor type	Conditioning regimen	Use of GvHD prophylaxis	Prophylactic antibiotics during conditioning	Engraftment day of WBC	Use of antibiotics at fecal sampling	
								before HSCT	after HSCT
1	M/5y6m	HLH reactivation	Haploidentical	rATG+Fludarabine +Treosulfan+TT	No	Yes (cefepime, D−8~D0)	+10	Yes (cefepime + ciprofloxacin)	No
2	F/19y	Relapsed ALL	Haploidentical	rATG+Fludarabine +TBI+CPM+IT MTX	No	Yes (cefepime, D−12~D0)	+10	No	No
3	F/14y11m	AML	Sibling	rATG+BU+CPM +IT AraC	Cys	No	+12	Yes (cefepime)	No
4	M/6y	AML	Haploidentical	rATG+Fludarabine +TBI+CPM+IT AraC	No	Yes (cefepime + metronidazole, D−10~D0)	+10	Yes (vancomycin + cefepime)	Yes (ciprofloxacin + cefepime)
5	M/16y11m	NK/T cell lymphoma with HLH	Haploidentical	rATG+Fludarabine +TBI+CPM+IT MTX	No	Yes (teicoplanin + cefepime, D−10~D0)	+10	Yes (cefditoren)	No
6	F/1y1m	Diamond−Blackfan syndrome	Sibling	rATG+Fludarabine + Treosulfan+TT	Cys +MMF	Yes (cefepime, D−9~D0)	+10	No	Yes (cefepime)
7	F/13y1m	ALL	Sibling	rATG+TBI+CPM +IT MTX	Cys	No	+14	No	No
8	M/8y5m	T	Sibling	rATG+TBI+CPM	Cys	No	+10	No	No

		lymphoblastic lymphoma		+IT MTX					
9	M/14y5m	Myelodysplastic syndrome	Haploidentical	rATG+Fludarabien +TBI+CPM	MMF	Yes (cefepime, D−9~D0)	+10	No	No
10	F/7y7m	HLH reactivation	Sibling	rATG+Fludarabine +Treosulfan+TT	Cys +MMF	No	+10	No	No

ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CPM = cyclophosphamide; Cys = cyclosporine; F = female; rATG = gamma anti−thymocyte globulin; GvHD = graft versus host disease; HLH = hemophagocytic lymphohistiocytosis; IT = intrathecal; M = male; MTX = methotrexate; MMF = mycophenolate mofetil; TBI = total body irradiation; TT = thiotepa

2. Clinical outcomes including acute GvHD following HSCT

Among 10 recipients with follow-up for at least 6 months, 3 patients out of the 10 developed aGvHD within the first 100-day after HSCT (Table 3). One recipient had acute skin GvHD with stage 3 at 17 days after HSCT, and another recipient had acute skin GvHD with stage 2 at 27 days after HSCT. Both of them were treated with steroids (2 mg/kg/day). The other recipient developed acute gastrointestinal and skin GvHD with grade 3 (intestinal stage 3, skin stage 3) at 29 days after HSCT and received steroids, infliximab and tacrolimus.

All the recipients survived the follow-up period, however, one of them recurred his underlying disease at 6 months after HSCT. Four recipients had CMV reactivation and one recipient had transplant-associated thrombotic microangiopathy.

Table 3. Clinical outcomes including acute GvHD following HSCT

No.	Acute GvHD			Survival	Treatment associated morbidity
		Location	Day		
1	Yes (Grade 3)	GI stage 3 + Skin stage 3	+29	O	CMV pneumonitis
2	No			O	Transplant-associated thrombotic microangiopathy
3	No			O	
4	Yes (Grade 2)	Skin stage 3	+17	O	
5	No			O	
6	Yes (Grade 1)	Skin stage 2	+27	O	
7	No			O	CMV reactivation
8	No			O	
9	No			O	CMV reactivation
10	No			O	CMV reactivation

CMV = cytomegalovirus; HSCT = hematopoietic stem cell transplantation; GI = gastrointestinal; GvHD = graft versus host disease

3. Gut microbiome of recipients and donors

Ten fecal samples from recipients were collected before HSCT, 10 after HSCT, 10 from the donor, and a total of 30 fecal samples were collected. The median day of pre-HSCT fecal sampling of recipients and post-HSCT fecal sampling was 18 days before HSCT (range, 6-52 days) and 34 days after HSCT (range, 26-63), respectively. The median day of donors' fecal sampling was 18 days before HSCT of relevant recipient (range, 0-52 days).

Four recipients received systemic antibiotics when collecting fecal samples before HSCT, while 2 recipients received intravenous antibiotics when collecting fecal samples after HSCT (Table 2). No difference in bacterial diversity in pre-HSCT gut microbiome and post-HSCT gut microbiome was observed with antibiotics use at the fecal sampling (Table 4). There was also no difference in bacterial diversity of post-HSCT gut microbiome according to the use of systemic antibiotics, except for antibiotics for gut decontamination, during conditioning.

Table 4 Comparison of bacterial diversity according to use of antibiotics

	Use of antibiotics at the fecal sampling before HSCT (n=4)	No use of antibiotics at the fecal sampling before HSCT (n=6)	*p*-value
Median Chao1 index of pre-HSCT gut microbiome	118.0261 (range, 55.8333-191.5)	202.9928 (range, 106.5882-289.1539)	0.171
Median Chao1 index of post-HSCT gut microbiome	88.0958 (range, 11.5-125.0625)	174.3286 (range, 89.4615-246.9231)	0.114
	Use of prophylactic antibiotics during conditioning (n=6)	No use of prophylactic antibiotics during conditioning (n=4)	*p*=value
Median Chao1 index of post-HSCT gut microbiome	107.262 (range, 11.5-178.5714)	160.7625 (range, 97-246.9231)	0.257

HSCT = hematopoietic stem cell transplantation

Microbiome composition of recipients and donors

Microbiome composition was investigated as follows (Figure 1). The dominant OTU at the phylum level in pre-HSCT gut microbiome of recipients was phylum *Bacteroidetes* in 5 recipients, phylum *Firmicutes* in 4 recipients, and phylum *Proteobacteria* in 1 recipient. The dominant OUT at the phylum level in post-HSCT gut microbiome of recipients was phylum *Firmicutes* in 7 recipients, phylum *Proteobacteria* in 2 recipients, and phylum *Actinobacteria* in 1 recipient. The gut microbiome of donors was dominated by several phyla including *Firmicutes* (7/10), *Proteobacteria* (1/10), *Bacteroidetes* (1/10), and *Actinobacteria* (1/10).

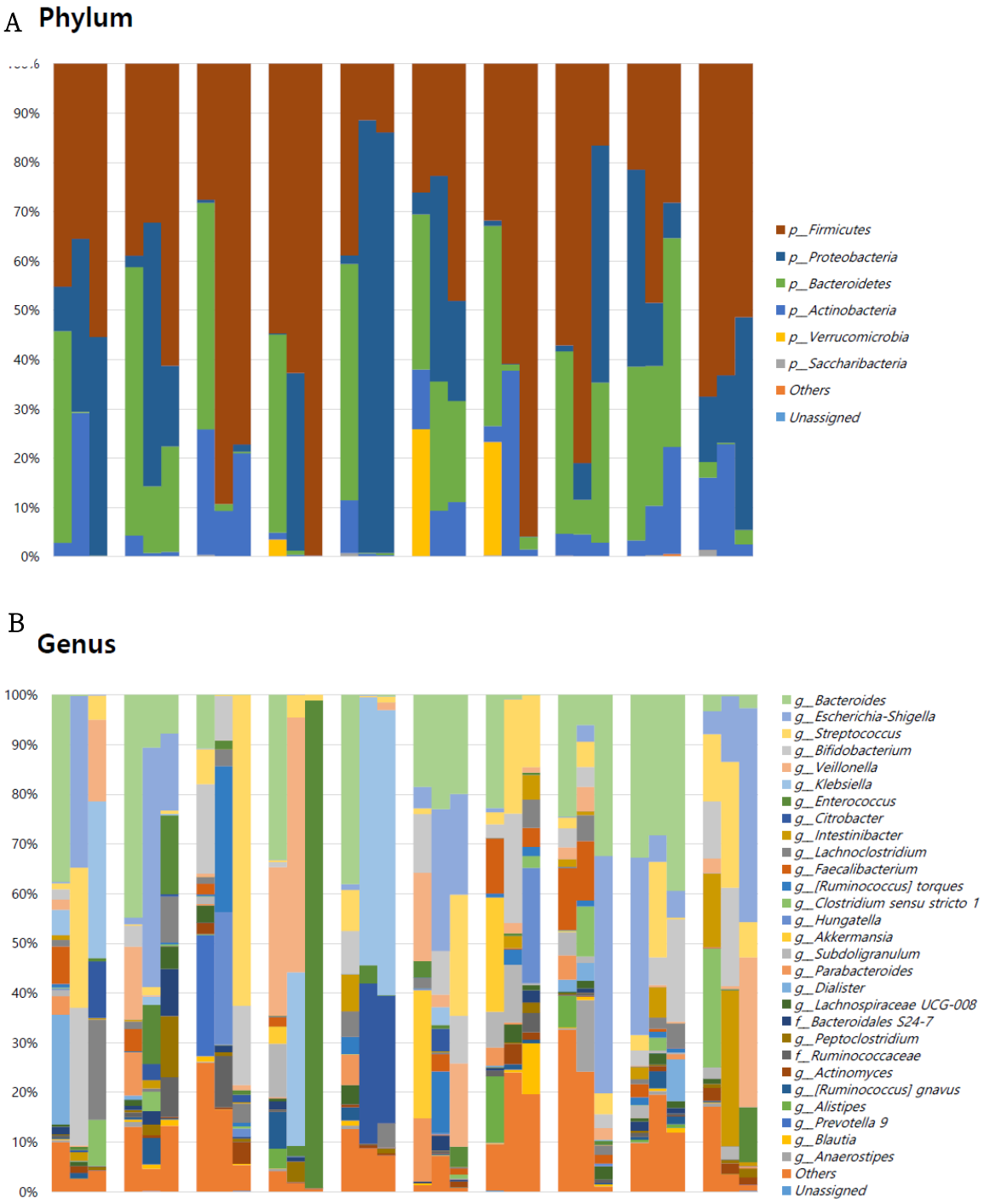


Figure 1. Gut microbiome composition. Each column represents the individual gut microbiome composition. Each bar in each column represents pre-HSCT gut microbiome of the recipient, post-HSCT gut microbiome of the recipient, and the relevant donor's gut microbiome in order A) at the phylum level, and B) at the genus level.

Bacterial diversity of gut microbiome of recipients and donors

We investigated the bacterial diversity, expressed as Chao1 index, in the pre-HSCT gut microbiome and in the post-HSCT gut microbiome of recipients (Figure 2). The median value of Chao1 index in pre-HSCT gut microbiome of recipients was 168.9821 (range 55.8333-289.1539). Chao1 index in post-HSCT gut microbiome of recipients (median 111.5938, range 11.5-246.9231) tended to show a lower diversity than one in pre-HSCT (p = 0.432) with loss of about 15% in average of the pre-HSCT gut microbiome. There was no statistically significant factor in univariate analysis conducted to elucidate the factors that reduce gut bacterial diversity (Table 5).

The median value of Chao1 index in donors' gut microbiome was 193.9624 (range 93.1538-322.0222). Although not statistically significant, a lower bacterial diversity was observed in pre-HSCT gut microbiome of recipients than in donors' gut microbiome (p = 0.105). The bacterial diversity of post-HSCT gut microbiome of recipients was lower than one of donors, either (p = 0.010).

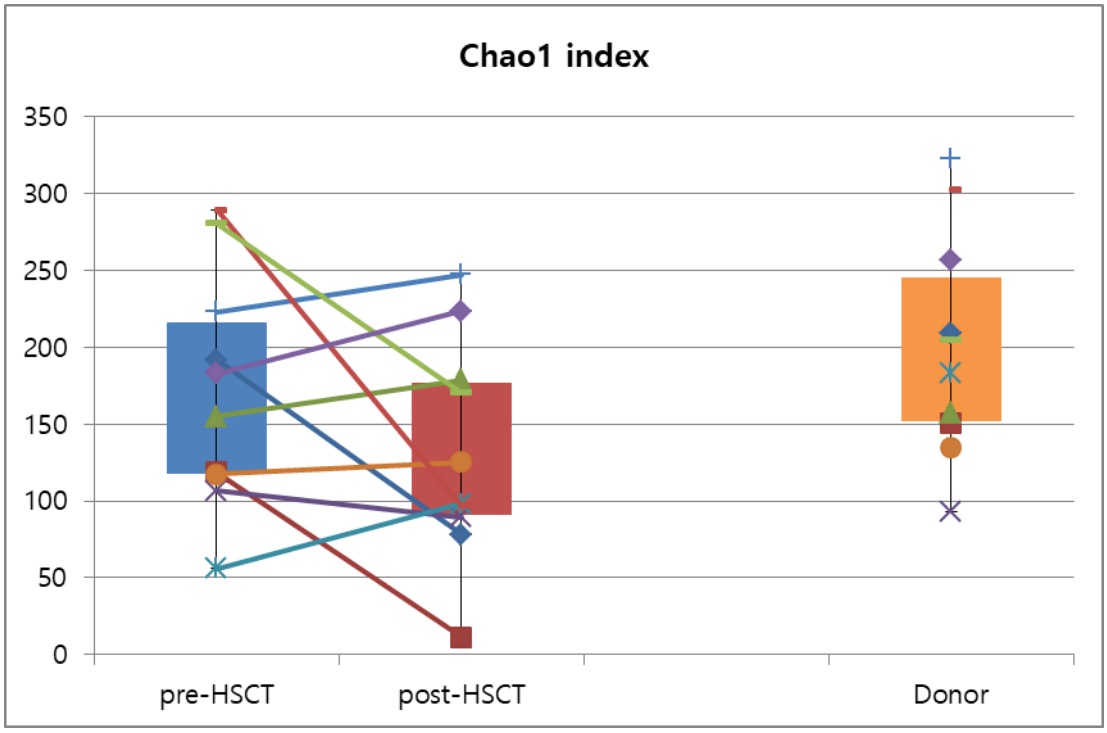


Figure 2. Gut bacterial diversity of recipients and donors. The gut bacterial diversity, expressed as Chao1 index, was reported as box plots.

Table 5. Factors associated with the decrease of bacterial diversity after HSCT of recipients

Variables	Odd ratio (95% confidence interval)
Median age at HSCT (months)	0.999 (0.981−1.018)
Underlying disease of malignancy	1.000 (0.045−22.175)
Donor type, Haploidentical	16.000 (0.722−354.803)
Conditioning regimen, Myeloablation	0.000 (0.000−∞)
Use of GvHD prophylaxis	0.167 (0.010−2.821)
Antibiotics at the time of fecal sampling before HSCT	1.000 (0.080−12.557)
Antibiotics at the time of fecal sampling after HSCT	1.000 (0.045−22.175)
Bacterial diversity of donor	0.996 (0.978−1.014)

HSCT = hematopoietic stem cell transplantation; GvHD = graft versus host disease

4. Acute GvHD and gut microbiome of recipients and donors

We found no significant differences between gut microbiome of recipient who developed aGvHD and recipients who did not develop aGvHD in terms of gender, the underlying disease, types of donor, conditioning regimens, engraftment day, use of GvHD prophylaxis and use of antibiotics at the fecal sampling. Recipients who experienced aGvHD were younger than recipients who did not experience aGvHD (Table 6).

Table 6. Comparison of demographic and clinical characteristics between recipients who developed acute GvHD and did not develop acute GvHD

Variables	acute GvHD (n=3)	No acute GvHD (n=7)	p-value
Sex, Male	2	3	1.000
Median age at HSCT (months)	66	173	0.017
Underlying disease of malignancy	2	6	1.000
Donor type, Haploidentical	2	1	1.000
Conditioning regimen, Myeloablation	0	3	0.475
Median day of engraftment (days)	10	10	0.533
Use of GvHD prophylaxis	1	5	0.500

HSCT = hematopoietic stem cell transplantation; GvHD = graft versus host disease

Gut bacterial diversity in relation to acute GvHD development

The median Chao1 index in post-HSCT gut microbiome of recipients who developed aGvHD was similar to those of recipients who did not develop

aGvHD (aGvHD 78.0667; no aGvHD 125.0625, p = 0.267) (Figure 3). The median Chao1 index in pre-HSCT sample of recipients who developed aGvHD was similar to those of recipients who did not develop aGvHD (aGvHD 155; no aGvHD 182.9643, p = 1.000). The median Chao1 index in donor's gut microbiome of recipients who developed aGvHD was similar to those of recipients who did not develop aGvHD (aGvHD 157.25; no aGvHD 204.8438, p = 0.667). The change in Chao1 index (increase or decrease) was not different between the two groups (p = 0.383).

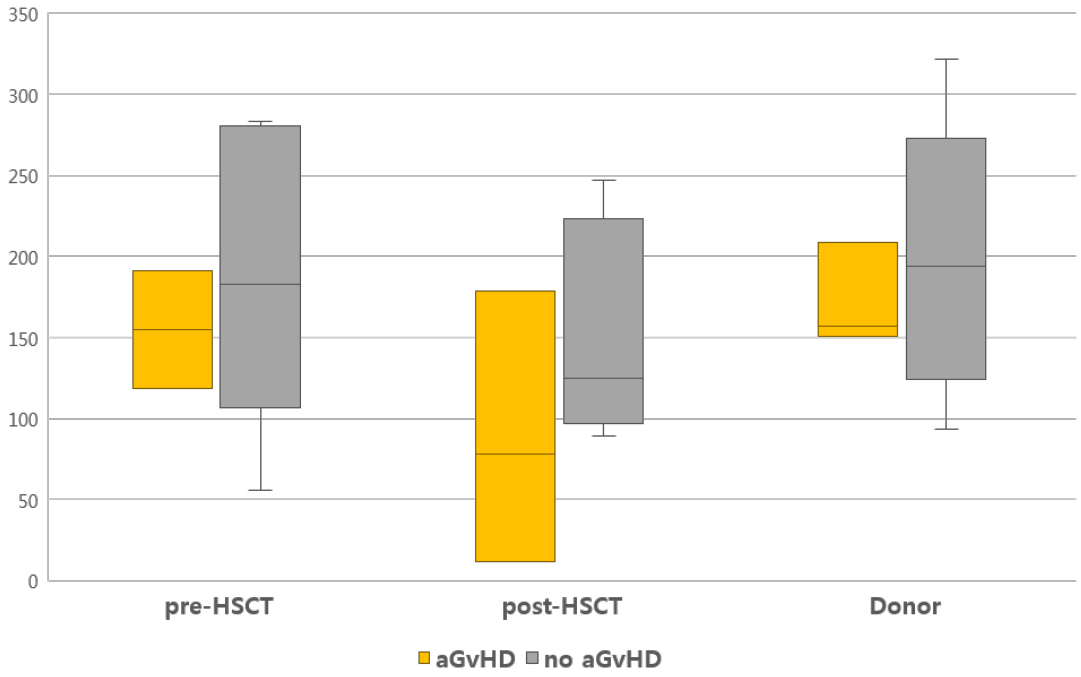


Figure 3. Comparison of gut bacterial diversity between recipients who developed acute GvHD and did not develop acute GvHD. The gut bacterial diversity, expressed as Chao1 index, was reported as box plots.

In patients who developed aGvHD, Chao1 index of post-HSCT gut

microbiome was lower than one in pre-HSCT gut microbiome with loss of about 43% in average of the pre-HSCT gut microbiome (p = 0.500) (Figure 4). Chao1 index of post-HSCT gut microbiome of recipients was lower than one of donors, either (p = 0.500).

In patients who did not develop aGvHD, Chao1 index of post-HSCT gut microbiome was lower than one in pre-HSCT gut microbiome with loss of about 17% in average of the pre-HSCT gut microbiome (p = 0.938). Chao1 index of post-HSCT gut microbiome of recipients was lower than one of donors, either (p = 0.016).

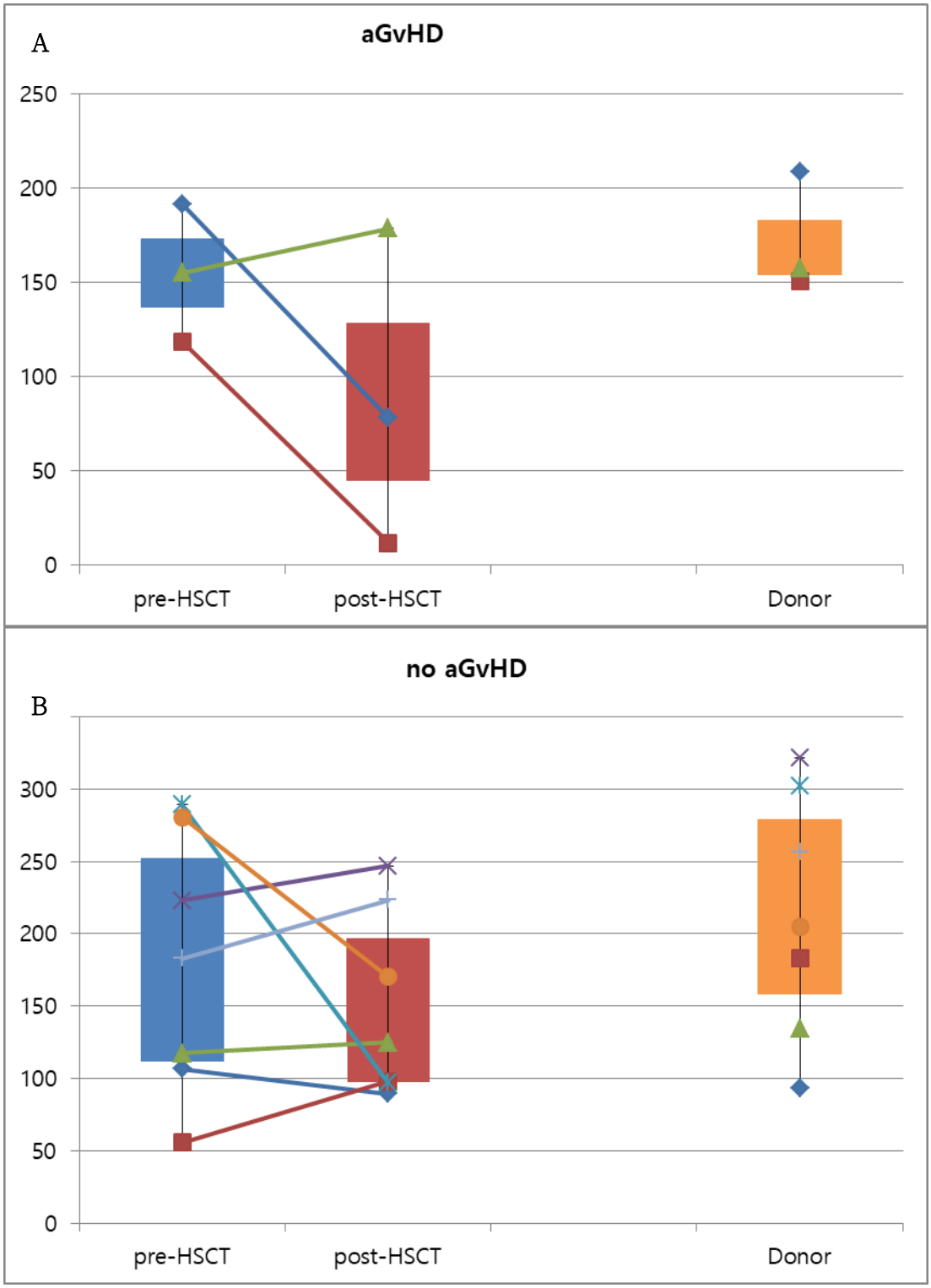


Figure 4. Dynamics of gut bacterial diversity A) in patients who developed acute GvHD, and B) in patients who did not develop acute GvHD.

Analysis of microbiome variability in relation to aGvHD development

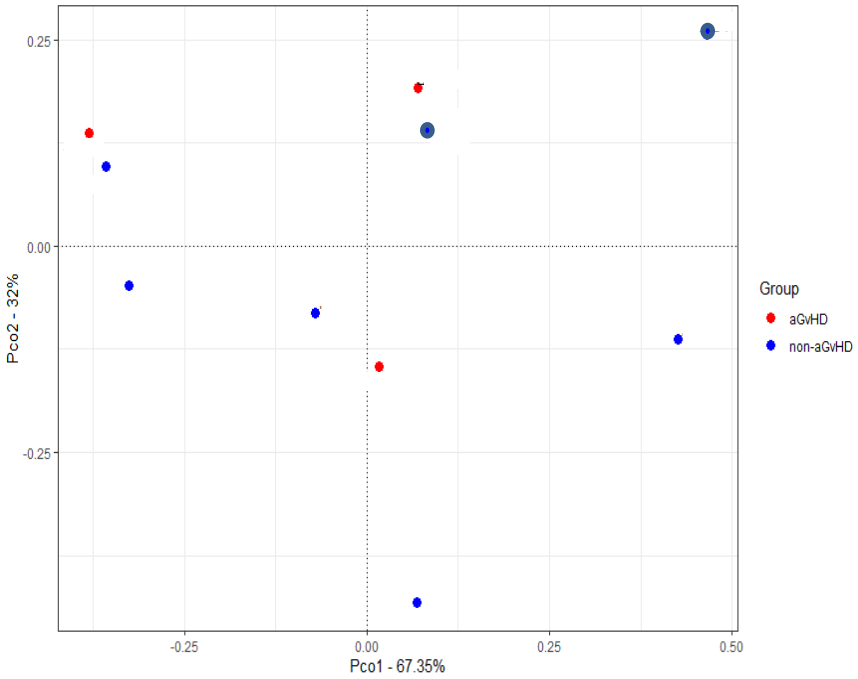
PCA based on phylum-level relative abundances and genus-level relative abundances showed no separation between gut microbiome collected after HSCT from recipients who did and did not develop aGvHD ($p > 0.05$) (Figure 5A, 5B). However, post-HSCT gut microbiome of recipients who developed aGvHD showed higher abundance of *Gerbera hybrid cultivar* genus ($p = 0.0345$) and lower abundance of *Erysipelatoclostridium* genus ($p = 0.0456$) in the composition of microbiome than one of recipients who did not develop aGvHD. The composition of microbiome at the phylum level did not differ between recipients who developed aGvHD and who did not develop aGvHD.

PCA also showed no separation between gut microbiome collected before HSCT from recipients who did and did not develop aGvHD ($p > 0.05$) (Figure 5C, 5D). There were no significant differences in the composition of microbiome before HSCT at the genus level and the phylum level.

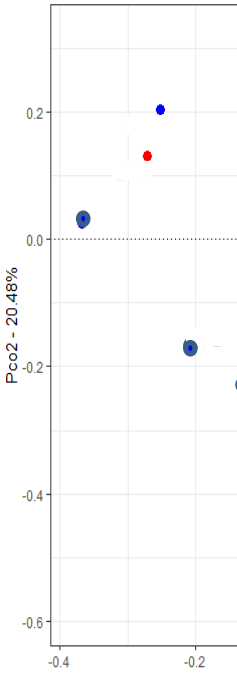
In donors' gut microbiome, PCA showed no separation from recipients who subsequently either did or did not develop aGvHD ($p > 0.05$) (Figure 5E, 5F). However, relevant donors' gut microbiome of recipients who developed aGvHD tended to show higher relative abundance of OTUs assigned to [Eubacterium] *eligens group* genus ($p = 0.040$) compared to donors' gut microbiome of recipients who did not develop aGvHD. The composition of microbiome at the phylum level did not differ between recipient who experienced aGvHD and recipients who did not experienced

aGvHD.

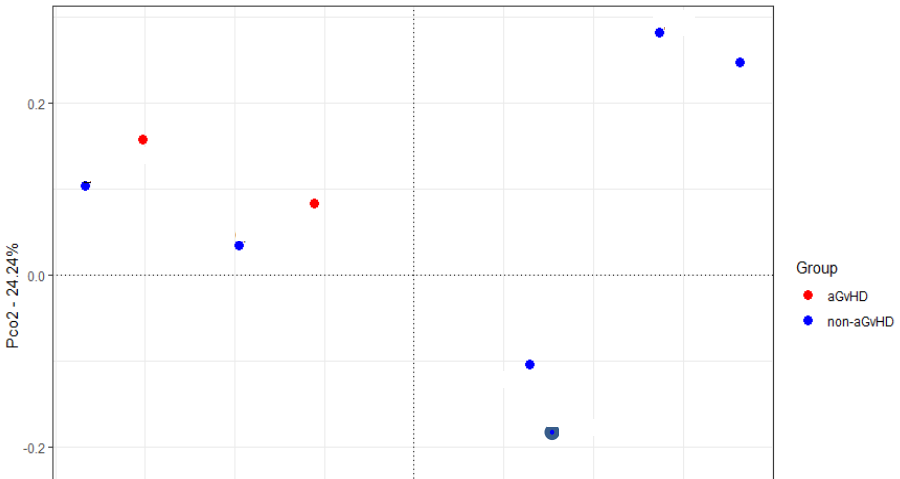
A


0.25
0.00
-0.25
Pco2 - 32%
-0.25
0.00
0.25
0.50
Pco1 - 67.35%
Group
aGvHD
non-aGvHD

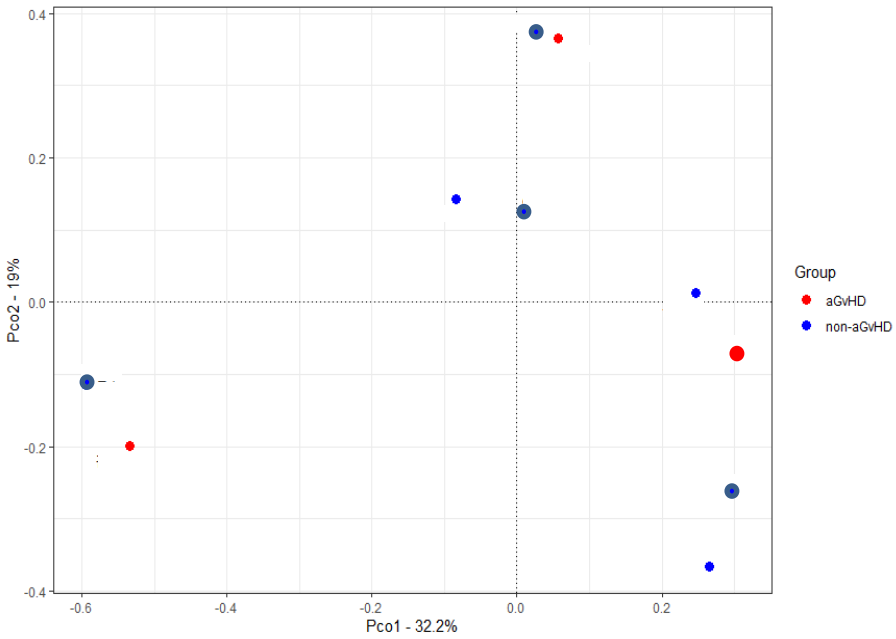
B


0.2
0.0
-0.2
-0.4
-0.6
Pco2 - 20.48%
-0.4
-0.2

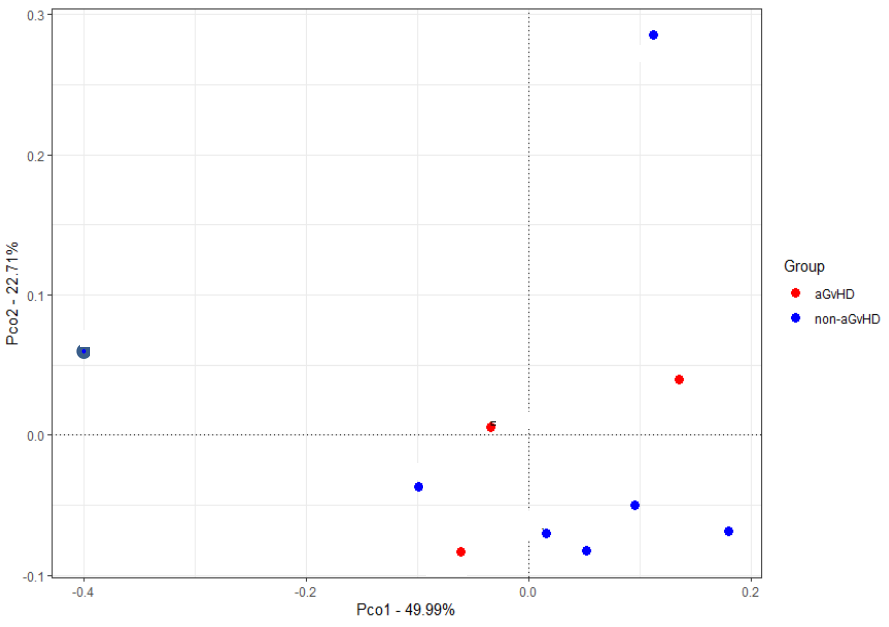
C


0.2
0.0
-0.2
Pco2 - 24.24%
Group
aGvHD
non-aGvHD

D


0.4
0.2
0.0
-0.2
-0.4
Pco2 - 19%
-0.6
-0.4
-0.2
0.0
0.2
Pco1 - 32.2%
Group
aGvHD
non-aGvHD

E


0.3
0.2
0.1
0.0
-0.1
Pco2 - 22.71%
-0.4
-0.2
0.0
0.2
Pco1 - 49.99%
Group
aGvHD
non-aGvHD

F

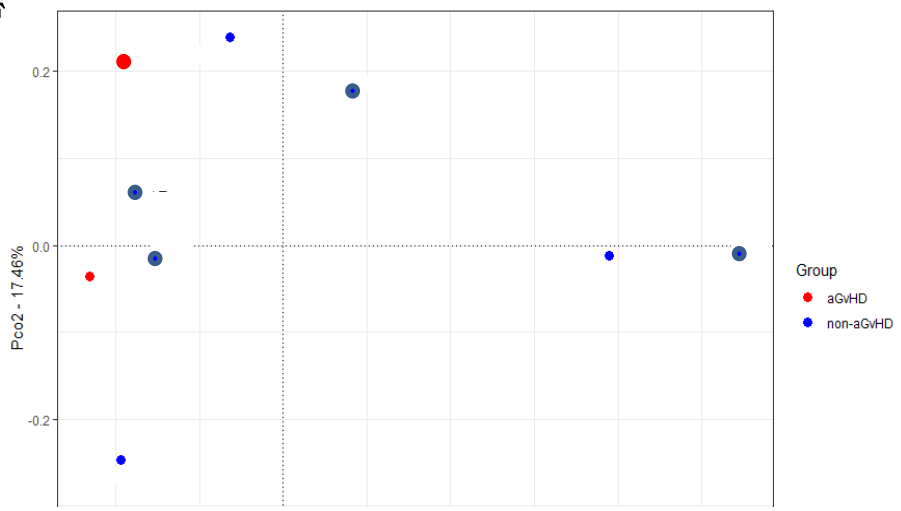


Figure 5. Gut microbiome variability in relation to acute GvHD development. PCA based on Bray-Curtis distances of A) phylum-level relative abundance profiles of feces collected after HSCT from patients who develop acute GvHD (red) and who did not develop acute GvHD (blue). B) genus-level relative abundance profiles of feces collected after HSCT, C) phylum-level relative abundance profiles of feces collected before HSCT, D) genus-level relative abundance profiles of feces collected before HSCT, E) phylum-level relative abundance profiles of donors' feces, F) genus-level relative abundance profiles of donors' feces.

The fSCT

Age at HSCT, the underlying disease, types of donor, conditioning regimens, use of GvHD prophylaxis, engraftment day, Chao1 index of pre-HSCT and post-HSCT in recipients, Chao1 index of donor, use of antibiotics at the time of fecal sampling before HSCT and after HSCT, relative abundance of *Erysipelatoclostridium* in the pre-HSCT gut microbiome, and *Erysipelatoclostridium* in the donors' gut microbiome were not associated with lower abundance of genus *Erysipelatoclostridium* in the univariate analysis (Table 7).

Table 7. Factors associated with *Erysipelatoclostridium* in post-HSCT gut microbiome

HSCT = hematopoietic stem cell transplantation; GvHD = graft versus host disease

Variables	Odd ratio (95% confidence interval)
Age at HSCT	2.421 (0.000−0.000418)
Underlying disease of malignancy	1.667 (0.074−37.728)
Donor type, Haploidentical	1.000 (0.080−12.557)
Conditioning regimen, Myeloablation	0.002154 (0.000−∞)
Use of GvHD prophylaxis	2.000 (0.150−26.734)
Engraftment day	20148.9 (0.000−∞)
Pre−HSCT Chao1 index	1.003 (0.986−1.021)
Post−HSCT Chao1 index	1.003 (0.985−1.022)
Donor Chao1 index	1.003 (0.984−1.021)
Use of antibiotics at the fecal sampling before HSCT	0.500 (0.037−6.683)
Use of antibiotics at the fecal sampling after HSCT	0.000 (0.000−∞)
Erysipelatoclostridium before HSCT	0.000 (0.000−0.000396)
Erysipelatoclostridium of donor	0.000 (0.000−∞)

5. CMV reactivation and gut microbiome of recipients and donors

We investigated the association between aGvHD development and bacterial diversity of recipients and their donors. The median Chao1 index in post-HSCT gut microbiome of recipients who had CMV reactivation was 196.7429 (range, 78.0667-246.9231). Although not statistically significant, Chao1 index in post-HSCT gut microbiome of recipients who had CMV reactivation tended to show a higher diversity compared to recipients who did not have CMV reactivation (median 97.5625, range 11.5-178.5714, p = 0.257) (Figure 6). The median Chao1 index in pre-HSCT gut microbiome of recipients who had CMV reactivation was 207.2606 (range, 182.6943-280.5652). The Chao1 index in pre-HSCT gut microbiome of recipients who had CMV reactivation tended to show a higher diversity compared to recipients who did not have CMV reactivation (median 118.0261, range 55.8333-289.1539, p = 0.114). The median Chao1 index in gut microbiome of donors whose relevant recipients experienced CMV reactivation was 232.5668 (range, 204.8438-322.0222). There was no significant difference in bacterial diversity between donors whose relevant recipients experienced CMV reactivation and donors whose relevant recipients did not experience (median 153.9821, range 93.1539-301.9355, p = 0.067).

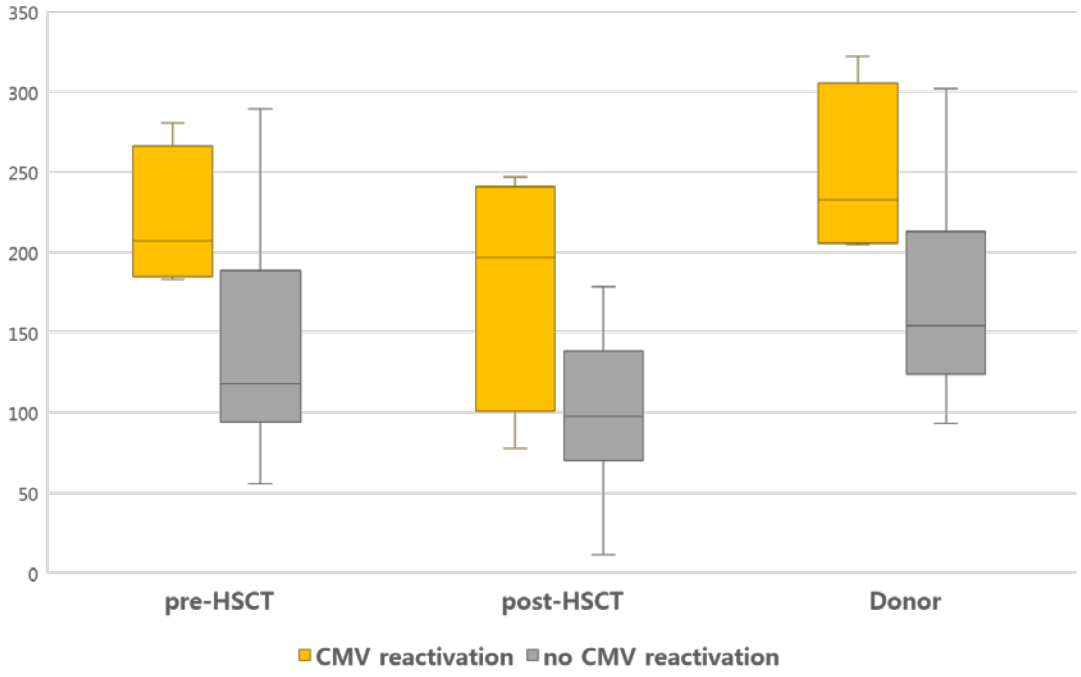


Figure 6 Comparison of gut bacterial diversity between recipients who experienced CMV reactivation and who did not experience CMV reactivation. The gut bacterial diversity, expressed as Chao1 index, was reported as box plots.

Discussion

According to our findings, Chao1 index in post-HSCT gut microbiome of

recipients tended to show a lower diversity by 15% compared to pre-HSCT gut microbiome of recipients. Although not statistically significant, the median Chao1 index in pre-HSCT gut microbiome of recipients, post-HSCT gut microbiome of recipients, and donor's gut microbiome in whom developed aGvHD was lower than those in pre-HSCT gut microbiome, post-HSCT gut microbiome, and donor's gut microbiome in whom did not develop aGvHD. Post-HSCT gut microbiome of recipients who developed aGvHD showed higher abundance of *Gerbera hybrid cultivar* genus and lower abundance of *Erysipelatoclostridium* genus in the composition of microbiome than one of recipients who did not develop aGvHD. Donors whose relevant recipients experienced aGvHD showed higher abundance of [Eubacterium] *eligens group* genus compared to donors whose relevant recipients did not experience aGvHD.

It has been reported the gut microbiome structure before HSCT of recipients is approximate to one of the healthy people, in terms of richness and diversity.[9-11, 26] In our study, we found a lower bacterial diversity was observed in pre-HSCT gut microbiome of recipients than in donors' sample who were considered as healthy. Gut microbiome depends on age and is affected by the various drugs including antibiotics, disease and diet. Five of donors in our study were adults. Four recipients used systemic antibiotics when fecal samples were taken. Eight recipients were treated for chemotherapy because of underlying disease before HSCT. The difference in bacterial diversity between pre-HSCT gut microbiome and donor's gut

microbiome could be explained by these factors.

In donors, the intestinal bacteria and their products influenced the activation and differentiation of immune cell populations like regulatory T cells. This influence occurs not only in the gut, but also in distant sites including the bone marrow.[27-29] Different donor microbiome may promote different compositions of HSCT donor immune cells, theoretically. A study of the association between the gut microbiome of donor and aGvHD in adult cohort by Liu et al. showed that high bacterial diversity in gut microbiome of donor decreased acute gastrointestinal GvHD risk (Odd ratio [OR] = 0.12, p = 0.038).[30]

We have shown that the difference in bacterial diversity of donors was not observed between recipients with aGvHD and without aGvHD in pediatric cohort study. In our study, donors whose relevant recipients experienced aGvHD showed higher abundance of [Eubacterium] *eligens group* genus compared to donors whose relevant recipients did not experience aGvHD. *Eubacterium* genus is one of commensal bacteria living in colon, and produces short chain fatty acid (SCFA) which is the most reliable biomarker of microbiota-host mutualistic configuration.[31] SCFAs are of particular interest for maintaining host health because they may exert anti-inflammatory effects through several mechanisms, including epithelial integrity (preserving tight junctions) and maintenance of the mucus layer.[32] It is not yet known whether *Eubacterium* genus affect the differentiation in

T cell in blood, or in turn, how it affects the occurrence of aGvHD in recipients. Further investigation is needed on the effect of donor's gut microbiome on donor immune cells and, by extension, donor immune cells on the occurrence of aGvHD of HSCT recipients.

Regarding pre-HSCT bacterial composition in allo-HSCT recipients, Biagi et al. reported lower abundance of two members of the phylum *Bacteroidetes* (*Bacteroides* and *Parabacteroides*) and higher abundance of phylum *Firmicutes* were observed in pre-HSCT gut microbiome in pediatric recipients with aGvHD, compared to pediatric recipients without aGvHD.[15] Likewise, Doki et al. reported that pre-HSCT gut microbiota from adult recipients with aGvHD showed a significantly higher abundance of phylum *Firmicutes* and a tendency for a lower abundance of *Bacteroidetes* than adult recipients without aGvHD.[14] However, we did not find the association between pre-HSCT gut microbiome and aGvHD. Another study by Liu et al. also did not show the association between pre-HSCT gut microbiome and aGvHD.[30] Further studies about the pre-HSCT gut microbiome are needed, given that pre-HSCT microbiome may contribute immunomodulatory or mucosal integrity functions that reduce future aGvHD risk.

The bacterial diversity in post-HSCT sample of recipients with aGvHD tended to be lower than in recipients without aGvHD in our study. Comprehensive studies demonstrated that lower bacterial diversity after HSCT and change in gut microbiome were contributed to occurrence of

aGvHD following HSCT. Increased amounts of the genus *Blautia*, which was one of commensal bacteria, was associated with reduced GVHD lethality.[13] A relative shift toward *Enterococci* was particularly prominent in patients that developed acute gastrointestinal GvHD.[9] A drop in the abundance of the known health-promoting *Faecalibacterium* and high percentages of *Enterococcus* was associated with aGvHD onset.[10] Post-HSCT samples of aGvHD patients showed lower abundance of *Erysipelatoclostridium* genus in the composition of microbiota in our study. *Erysipelatoclostridium* genus is considered as human opportunistic pathogens in the human gut and known to be possible to influence allergic disease in children, gout and metabolic syndrome in adults through their metabolites.[33-35] *Erysipelatoclostridium* genus increased dramatically in a Crohn's disease patients.[36] The up-regulation of opportunistic pathogens in the human gut might contribute to change to intestinal barrier function and change to immune tolerance to intestinal antigens, thus, affect occurrence of disease.[37] However, in aGvHD, the opposite pattern was observed and further investigation is required.

The use of broad-spectrum antibiotics, which is necessary to treat neutropenic infection that occur during the HSCT process, may exert a detrimental impact on intestinal microbiota composition and, subsequently, the occurrence of aGvHD. The antibiotic use after HSCT worsened loss of microbiome diversity, loss of intestinal commensals that produce SCFAs, and increased risk of GvHD.[9,38] The decline in anti-inflammatory Clostridia in the gut microbiota was associated with the development of GvHD in

pediatric HSCT patients.[39] Antibiotics with reduced activity against anaerobic commensal bacteria, such as piperacillin-tazobactam or imipenem-cilastatin was associated with aggravated gut microbial perturbation and a significantly higher GvHD-related mortality.[40] The use of antibiotics before HSCT was further associated with a higher GvHD, as well as the use of antibiotics after HSCT.[41] Unlike previous studies, the use of antibiotics before HSCT and after HSCT did not affect GvHD in our study (use of antibiotics before HSCT, OR = 5.000, 95% confidence interval [CI] 0.273-91.518; use of antibiotics HSCT, OR = 0.000, 95% CI 0.000-∞). It is difficult to compare our study with the previous study results because there was small number of patients in our study. Further studies among pediatric patients with use of various antibiotics are needed.

Recently, attempts to manipulation of the gut microbiome before or after HSCT as a therapeutic potential for aGvHD are under way. Potential interventions range from the use of prebiotic, probiotics, diet and fecal microbiome transfer (FMT), to the targeted antibiotic therapies tailored on the composition of the gut microbiome. They could modify the composition of the gut microbiome and thereby mediate anti-inflammatory effects. Recent studies showed the feasibility of FMT for steroid refractory gastrointestinal GvHD, especially.[42-43] FMT consists of the introduction of a fecal suspension derived from a healthy donor. Even though the studies involved a small number of patients, all patients relieved their symptoms without severe adverse events. Our results regarding the association of gut

microbiome of recipients and donors with aGvHD are thought to be help in choosing the donor of FMT.

This study has several limitations. First, the number of enrolled patients and the number of fecal samples are small. Therefore, there was a limit to identifying the factors that caused the occurrence of aGvHD or microbial changes. Second, gut microbiome in HSCT recipients is affected by diet and conditioning regimen. Thus, detailed studies are needed, given that Koreans have different diets from the West and that the conditioning regimen are different by donor type of HSCT or underlying disease of recipients. Third, the mechanism in which the gut microbiome difference between the recipients with aGvHD and the recipients without aGvHD cause disease has not been identified. It is not yet clear whether dysbiosis of the gut microbiome is a cause or consequence of aGvHD in humans, although there is a lot of study on association between aGvHD and gut microbiome. It is necessary to confirm whether the differences in microbial composition observed in our study are significant, by identifying the change of their metabolites, such as SCFAs.

Conclusion

Chao1 index in post-HSCT gut microbiome of recipients tended to show a

lower diversity by 15% compared to pre-HSCT gut microbiome of recipients. Although not statistically significant, the median Chao1 index in pre-HSCT gut microbiome of recipients, post-HSCT gut microbiome of recipients, and donor's gut microbiome in whom developed aGvHD was lower than those in pre-HSCT gut microbiome, post-HSCT gut microbiome, and donor's gut microbiome in whom did not develop aGvHD, respectively. Post-HSCT gut microbiome of recipients who developed aGvHD showed higher abundance of *Gerbera hybrid cultivar* genus and lower abundance of *Erysipelatoclostridium* genus in the composition of microbiome than one of recipients who did not develop aGvHD. Donors whose relevant recipients experienced aGvHD showed higher abundance of [Eubacterium] *eligens group* genus compared to donors whose relevant recipients did not experience aGvHD. Our data suggested that the gut microbiome of pediatric recipients after HSCT and donor's gut microbiome showed difference between recipients who developed aGvHD and recipients who did not develop aGvHD. Identifying the gut microbiome of recipients and donors could help predict the occurrence of aGvHD.

References

1. Knight, R. and B. Buhler, Follow your gut: the enormous impact of tiny microbes. First TED Books hardcover edition. 2015. 114 pages

2. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007;449:804-10.

3. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2007;2:204.

4. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 2010;330:1768-73.

5. Candela M, Turroni S, Biagi E, Carbonero F, Rampelli S, Fiorentini C, et al. Inflammation and colorectal cancer, when microbiota-host mutualism breaks. World J Gastroenterol 2014;20:908-22.

6. Duvallet C, Gibbons SM, Gurry T, Irizarry RA, Alm EJ. Meta-analysis of gut microbiome studies identifies disease-specific and shared responses. Nat Commun 2017;8:1784.

7. Nieuwdorp M, Gilijamse PW, Pai N, Kaplan LM. Role of the microbiome in energy regulation and metabolism. Gastroenterology 2014;146:1525-33.

8. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet 2009;373:1550-61.

9. Holler E, Butzhammer P, Schmid K, Hundsrucker C, Koestler J, Peter K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. Biol Blood Marrow Transplant 2014;20:640-5.

10. Biagi E, Zama D, Nastasi C, Consolandi C, Fiori J, Rampelli S, et al. Gut microbiota trajectory in pediatric patients undergoing hematopoietic SCT. Bone Marrow Transplant. 2015;50:992-8.

11. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood 2014;124:1174-82.

12. Weber D, Oefner PJ, Hiergeist A, Koestler J, Gessner A, Weber M, et al. Low urinary indoxyl sulfate levels early after transplantation reflect a disrupted microbiome and are associated with poor outcome. Blood 2015;126:1723-28.

13. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal Blautia Is Associated with Reduced Death from Graft-versus-Host Disease. Biol Blood Marrow Transplant 2015;21:1373-83.

14. Doki N, Suyama M, Sasajima S, Ota J, Igarashi A, Mimura I, et al. Clinical impact of pre-transplant gut bacterial diversity on outcomes of allogeneic hematopoietic stem cell transplantation. Ann Hematol 2017; 96:

1517-23.

15. Biagi E, Zama D, Rampelli S, Turroni S, Brigidi P, Consolandi C, et al. Early gut microbiota signature of aGvHD in children given allogeneic hematopoietic cell transplantation for hematological disorders. BMC Med Genomics. 2019;12:49.

16. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 2013;500:232-6.

17. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. Proc Natl Acad Sci USA 2010; 107: 12204-9.

18. Furusawa Y, Obata Y, Hase K. Commensal microbiota regulates T cell fate decision in the gut. Semin Immunopathol 2015; 37:17-25.

19. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974; 18: 295-304.

20. Martin M. Cut adapt removes adapter sequences from high-throughput sequencing reads. EMBnet journal 2011;17:10-2.

21. Kwon S, Lee B, Yoon S. CASPER: context-aware scheme for paired-end reads from high-throughput amplicon sequencing. BMC bioinformatics

2014:15;S10.

22. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, et al. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nature methods 2013;10:57.

23. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: a versatile open source tool for metagenomics. PeerJ 2016;4:e2584.

24. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research 2012;41:D590-6.

25. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods 2010;7:335-6.

26. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science 2005;308:1635-38.

27. Khosravi A, Yáñez A, Price JG, Chow A, Merad M, Goodridge HS, et al. Gut

microbiota promote hematopoiesis to control bacterial infection. Cell Host Microbe 2014;15:374-81.

28. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N,

Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. Nat Med 2014;20:159-66.

29. Josefsdottir KS, Baldridge MT, Kadmon CS, King KY. Antibiotics impair murine hematopoiesis by depleting intestinal microbiota. Blood 2017;129:729-39.

30. Liu C, Frank DN, Horch M, Chau S, Ir D, Horch EA, et al. Associations between acute gastrointestinal GvHD and the baseline gut microbiota of allogeneic hematopoietic stem cell transplant recipients and donors. Bone Marrow Transplant 2017;52:1643-50.

31. Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, et al. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. Isme J 2014;8:1323-35.

32. Macia L, Thorburn AN, Binge LC, Marino E, Rogers KE, Maslowski KM, et al. Microbial influences on epithelial integrity and immune function as a basis for inflammatory diseases. Immunol Rev 2012;245:164-76.

33. Shen X, Wang M, Zhang X, He M, Li M, Cheng G, et al. Dynamic construction of gut microbiota may influence allergic diseases of infants in Southwest China. BMC Microbiol 2019;19:123.

34. Shao T, Shao L, Li H, Xie Z, He Z, Wen C. Combined Signature of the Fecal Microbiome and Metabolome in Patients with Gout. Front Microbiol 2017;8: 268.

35. Smith-Brown P, Morrison M, Krause L, Davies PS. Dairy and plant based food intakes are associated with altered faecal microbiota in 2 to 3 year old Australian children. Sci Rep 2016;6:32385.

36. Qiu Z, Yang H, Rong L, Ding W, Chen J, Zhong L. Targeted Metagenome Based Analyses Show Gut Microbial Diversity of Inflammatory Bowel Disease patients. Indian J Microbiol 2017;57:307-15.

37. Peled JU, Hanash AM, Jenq RR. Role of the intestinal mucosa in acute gastrointestinal GVHD. Blood 2016;128:2395-402.

38. Romick-Rosendale LE, Haslam DB, Lane A, Denson L, Lake K, Wilkey A, et al. Antibiotic Exposure and Reduced Short Chain Fatty Acid Production after Hematopoietic Stem Cell Transplant. Biol Blood Marrow Transplant 2018;24:2418-24.

39. Simms-Waldrip TR, Sunkersett G, Coughlin LA, Savani MR, Arana C, Kim J, et al. Antibiotic-Induced Depletion of Anti-inflammatory Clostridia Is Associated with the Development of Graft-versus-Host Disease in Pediatric Stem Cell Transplantation Patients. Biol Blood Marrow Transplant 2017;23:820-9.

40. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med 2016;8:339.

41. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota Disruption Induced by Early Use of Broad-Spectrum Antibiotics Is an Independent Risk Factor of Outcome after Allogeneic Stem Cell Transplantation. Biol Blood Marrow Transplant 2017;23:845-52.

42. Kakihana K. Fecal microbiota transplantation for acute graft-versus-host disease of the gut. Rinsho Ketsueki 2017;58:499-505.

43. Qi X, Li X, Zhao Y, Wu X, Chen F, Ma X, et al. Treating Steroid Refractory Intestinal Acute Graft-vs.-Host Disease With Fecal Microbiota Transplantation: A Pilot Study. Front Immunol 2018;9:2195.

국문초록

목적: 급성 이식편대숙주병은 동종 조혈모세포이식 후 공여자 유래 T 림프구가 수여자의 조직을 비자신의 조직으로 인식하여 발생하는 면역학적 질병이다. 위장관 마이크로바이옴의 불균형은 급성이식편대숙주병과 같은 염증상 질병을 유발할 수 있는 것으로 알려져 있다. 그러나 소아 조혈모세포이식에서 급성 이식편대숙주병의 발생과 공여자 및 수여자의 위장관 마이크로바이옴과의 연관성은 아직 잘 알려지지 않았다. 이 연구는 소아 조혈모세포이식에서 급성 이식편대숙주병의 발생과 공여자 및 수여자의 위장관 마이크로바이옴과의 연관성을 알아보고자 하였다.

방법: 이 연구는 전향적 연구로서 2018년 9월부터 2019년 2월까지 서울 아산병원에서 동종 조혈모세포이식을 받은 19세 이하의 소아청소년을 대상으로 하였다. 연구자들은 수여자로부터 조혈모세포이식 전의 대변과 조혈모세포이식 후의 1개월째의 대변 공여자로부터 관계가 있는 수여자의 조혈모세포 이식 전의 대변을 수집하였다. 대변의 미생물 분석은 16S rDNA 유전자 배열을 이용하였다. 조작분류단위를 기반으로 한 세균 다양성은 Caho1 지표를 계산하여 평가하였고 각각의 검체 사이에서 다양성의 비교는 Bray-Curtis 유사도를 사용하였다. 연구에 등록된 환자들의 인구학적 특징과 임상적 특징은 의무 기록을 통해 확인하였다. 급성 이식편대숙주병은 International Bone Marrow Transplant Registry 기준에 따라 임상적으로 진단하여 등급을 분류하였다.

결과: 총 10명의 소아 조혈모세포이식 환자가 연구에 등록되었다. 수여자의 조혈모세포이식 전 Choa1 지표의 중앙값은 168.9821 (range, 55.8333-289.1539) 이었고, 수여자의 조혈모세포 이식 후 Chao1 지표의 중앙값은 111.5938 (range, 11.5-246.9231) 이었다. 수여자의 이식 후 Chao1 지표는 이식 전과 비교하여 15% 감소하였다. 연구에 등록된 환자 중 3명이 이식 후 100일 이내에 급성 이식편대숙주병을 진단받았다. 2명은 각각 2단계와 3단계의 급성 피부 이식편대숙주병을 진단받았다. 나머지 1명은 3등급의 급성 위장관, 피부 이식편대숙주병을 진단받았다. 급성 이식편대숙주병이 발생한 수여자들의 Chao1 지표는 급성 이식편대숙주병이 발생하지 않은 수여자들에 비해 수여자의 이식 전, 수여자의 이식 후, 공여자 모두에서 낮았다. 하지만 통계적으로 유의한 차이는 없었다. 급성 이식편대숙주병이 발생한 수여자들은 급성 이식편대숙주병이 발생하지 않은 수여자들과 비교했을 때 이식 후 위장관 마이크로바이옴 중 *Gerbera hybrid cultivar* 종은 상대적으로 많았고 (p=0.0345) *Erysipelatoclostiridium* 종은 상대적으로 적었다(p=0.045). 또한 급성 이식편대숙주병이 발생한 수여자들은 그들의 공여자의 위장관 마이크로바이옴 중 [Eubacterium] *eligens group* 종이 상대적으로 많았다. 그러나 급성 이식편대숙주병이 발생한 수여자들과 발생하지 않은 수여자들 사이에서 이식 전 위장관 마이크로바이옴의 차이는 없었다.

결론: 조혈모세포이식을 받은 소아에서 급성 이식편대숙주병이 발생한 수여자와 급성 이식편대숙주병이 발생하지 않은 수여자에서 세균 다양성의 차이는 없었지만 수여자의 이식 후 위장관 마이크로바이옴과 공여자의 위장관

마이크로바이옴의 구성에서 차이를 보였다. 수여자와 공여자의 위장관 마이크로바이옴의 구성을 확인하는 것은 급성 이식편대숙주병의 발생을 예측하는데 도움이 될 수 있겠다.

중심단어 : 급성 이식편대숙주병, 동종 조혈모세포이식, 위장관 마이크로바이옴, 16S rDNA 유전자 배열 분석, 소아