International Journal for Vitamin and Nutrition Research

Int. J. Vitam. Nutr. Res. **2025**; 95(1): 26126
https://doi.org/10.31083/IJVNR26126

Original Communication

Changes in Vitamin D and Gut Microbiota in Pediatric Hematopoietic Stem Cell Transplantation Patients with Bloodstream Infections

Qian Gao[1], Mingjian Bai[1], Tianqi Qi[1], Jing Zhai[1], Yan Song[1], Weijie Zhang[2,*], Guowei Liang[1,*]

[1]Department of Clinical Laboratory, Aerospace Center Hospital, 100049 Beijing, China
[2]Department of Hematology, Aerospace Center Hospital, 100049 Beijing, China
*Correspondence: zhangweijie0626@sina.com (Weijie Zhang); LGW721@163.com (Guowei Liang)

Academic Editor: Torsten Bohn
Submitted: 14 August 2024 Revised: 11 November 2024 Accepted: 21 November 2024 Published: 12 February 2025

Abstract

Background: Vitamin D (VD) and gut microbiota (GM) are important variables in pediatric hematopoietic stem cell transplantation (HSCT) recipients with bloodstream infections (BSI). Both VD and GM play significant roles in immune regulation and in maintaining intestinal barrier function. **Methods**: This prospective case-control study included 48 consecutive pediatric patients who underwent HSCT, as well as 20 healthy children from the community. Plasma samples were collected pre- and post-HSCT, together with post-HSCT fecal samples. Serum 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$) were measured using chemiluminescence and enzyme linked immunosorbent assay, respectively. GM was analyzed by 16S rDNA next generation sequencing. **Results**: The incidence of BSI in pediatric HSCT recipients was 33.3% (16/48). No significant differences in serum 25(OH)D or $1,25(OH)_2D_3$ levels were observed between the BSI and non-BSI groups either before or after transplantation, or with the healthy control group. The α-diversity of GM in BSI and non-BSI patients was significantly lower than in healthy subjects. Proteobacteria were significantly more abundant in BSI patients than in non-BSI patients ($p = 0.0434$) or healthy controls ($p = 0.0193$). Pediatric HSCT patients showed significantly higher levels of *Staphylococcus* ($p < 0.001$), *Pseudomonas* ($p < 0.001$), *Enterococcus* ($p < 0.001$), *Clostridium innocuum* ($p = 0.0175$) and *Enterobacter* ($p = 0.0394$) compared to the controls, whereas the levels of Firmicutes ($p = 0.009$), Actinobacteria ($p < 0.001$), *Bifidobacterium* ($p < 0.001$) and *Faecalibacterium* ($p < 0.001$) were significantly lower. β-diversity analysis revealed significant population differences between the three groups. **Conclusions**: These results indicate there is no practical value in monitoring VD in HSCT patients. During HSCT and BSI, the GM experiences a loss of probiotics and an increase in potential pathogens.

Keywords: Vitamin D; gut microbiota; hematopoietic stem cell transplantation; bloodstream infections; sepsis

1. Introduction

Infection is a critical complication that affects the prognosis and survival of allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients, particularly in the pediatric population. The incidence of bloodstream infections (BSI) in pediatric HSCT recipients ranges from 10–50%, with an associated mortality rate of 5–15% [1,2]. BSI occurs mainly during the early pre-engraftment phase due to neutropenia and mucosal damage [3], which are inevitable in the post-transplant period. Vitamin D (VD) and gut microbiota (GM) were recently shown to play significant roles in immune regulation and in maintaining proper intestinal barrier function [4]. Further investigation of these two factors may provide novel insights into the prevention and management of pediatric HSCT patients with BSI.

Vitamin D3 is produced following solar UVB radiation on the skin, and is also absorbed directly by the gastrointestinal tract. It is first converted to 25-hydroxyvitamin D by the action of vitamin D-25-hydroxylase (25(OH)D) in the liver, and then to the bioactive form of 1,25-dihydroxyvitamin D3($1,25(OH)_2D_3$), or calcitriol, in the kidney. Beyond its impact on bone health, VD appears to play a vital role in the immune response to infection [5]. Studies have shown a correlation between VD deficiency and BSI or sepsis, both in adults [6,7] and children [8]. However, specific studies of child HSCT recipients have shown that VD deficiency is not associated with bacterial infection [9,10]. The association between VD deficiency and the prognosis of pediatric patients is also inconsistent [11,12].

The population of microorganisms that inhabit the gut is known as the GM. This plays a vital role in metabolizing energy, producing short chain fatty acids (SCFA) and vitamins, and regulating the immune system [13]. The stability of the intestinal environment and general well-being is maintained by a high abundance of GM with good composition, together with epithelial integrity and a strong immune system. These are altered during HSCT [14] and sepsis [15], and interactions may occur between VD, GM and sepsis [8,16,17], especially in children [13]. Study of the GM may result in a better understanding of the mechanism of gut-derived BSI and its prevention, leading to improved treatment of BSI with specific probiotics or with fecal microbiota transplantation (FMT).

Only a few studies have examined changes in the GM in HSCT children with BSI [18–20], and little is known about the role of VD. Therefore, the aim of this research was to evaluate VD and GM status in children who underwent allo-HSCT. These were compared between patients with or without BSI, and with healthy controls.

2. Materials and Methods

2.1 Study Design

We conducted a prospective case-control study between January 2022 and June 2023 in the hematology department of the Aerospace Center Hospital, a tertiary hospital in Beijing, China. Serum and stool samples were collected prospectively, together with clinical data including treatment and possible exposure factors. Patients were grouped according to the occurrence of BSI.

2.2 Participants

Fifty-five consecutive pediatric patients aged 4–17 years who underwent HSCT were enrolled in this study. Of these, 7 were excluded because they did not continue treatment at Aerospace Center Hospital. The final cohort of 48 patients were followed up from 7 days pre-HSCT to at least 100 days post-HSCT. Sixteen patients suffered a BSI during the period of granulocytopenia, as defined by the International Pediatric Sepsis Consensus Conference criteria [21]. BSI pathogens were identified by matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF, VITEK MS, IVD V3.2, bioMérieux, Marcy l'Etoile, France) after at least two vials of blood culture were deemed positive, or by metagenomic next-generation sequencing (Miniseq sequencing system, product No.SY-420-1001,Illumina, San Diego, CA, USA) of the peripheral blood. The control group consisted of 20 healthy juveniles with a similar gender and age profile and recruited from the community. Patient information on medication, invasive procedures, and complications was obtained from the electronic medical record, while results for complete blood cell count and biochemical tests were obtained from the clinical laboratory of the Aerospace Center Hospital. This study was approved by the Ethics Committee of the Aerospace Center Hospital (approval number: 2022065). All parents consented for storage of their children's blood and fecal specimens for use in the main trial, but not for future research.

2.3 Measurement of Vitamin D

Case and control groups were derived from the same prospective cohort, thus ensuring comparable baseline characteristics. However, in actual clinical practice some patients were observed to consume low oral doses of exogenous vitamin D3 (125 IU/day) or calcitriol (0.25 µg/day) to prevent steroid-related osteoporosis. To assess whether this direct supplementation had any impact on serum VD levels, the corresponding clinical information was also obtained. Peripheral blood samples were collected 7 days before conditioning, and 20 days post-HSCT. After centrifugation, plasma samples were stored at –20 ℃ for analysis of 25-hydroxyvitamin D using a chemiluminescence reagent (Mindary, Shenzhen, China, product lot 2022050122) and chemiluminescence immunoassay analyzer (CL6000i, Mindary, Shenzhen, China). 1,25-dihydroxyvitamin D was evaluated using the enzyme linked immunosorbent assay method (Dogesce, Beijing, China, product number DG12486H) and a microplate reader (SPARK version 2.0, Tecan Austria GmbH, Grödig, Austria, product number 16039400).

2.4 Next-generation Sequencing of Gut Microbiota

Fecal samples were collected 20 days post-HSCT for the patients, and on the day of recruitment for the normal control group. They were stored at –80 ℃ for no more than 6 months prior to analysis of the GM.

16S rDNA nucleic acid was extracted from at least 100 mg of stool sample using a QIAamp Fast DNA Stool Mini Kit (51604, QIAGEN, Hilden, North Rhine-Westphalia, Germany). The resulting template DNA samples had a qualified concentration of 24–1060 ng/µL and purity (A260/A280) of 1.683–1.781. The V3–V4 hypervariable region of 16S rDNA genes was amplified using universal primers with barcodes: forward (5′-3′), CCTACGGGRSGCAGCAG; reverse (5′–3′): GGACTACVVGGGTATCTAATC. The PCR products were detected by 2% agarose gel electrophoresis and purified using the Agencourt AMPure XP Nucleic Acid Purification Kit (A63881, Beckman Coulter, Brea, CA, USA). Prior to library preparation, a magnetic bead-based clean-up system was used to remove any joint self-contiguous segments. The library template was enriched by PCR using the KAPA HiFi Hotstart ReadyMix kit (KR0370, Kapa Biosystems, Roche, Basel, Switzerland). PCR products were detected by 2% agarose gel electrophoresis, and the AxyPrep DNA Gel Recovery Kit (08/05 Ver. 1, Axygen Biosciences, Union City, CA, USA) was used to recover PCR products after gel cutting. Following recovery, the library quality was evaluated using a Thermo NanoDrop 2000 UV spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) and 2% agarose gel electrophoresis. Finally, single-stranded DNA fragments were produced by denaturation with sodium hydroxide. The bridge PCR method was used for next-generation sequencing on the Illumina NovaSeq 6000 platform (20012850, Illumina, San Diego, CA, USA).

2.5 Bioinformatics Analysis

Clean tags were obtained after filtering for length (minimum/maximum = 200/550 bp) and quality (default parameters) using Mothor Software and Silva Gold Database. The 0.97 similarity uparse method and unoise3 method were used to cluster and de-noise the high-quality sequences, thereby obtaining several features. Operational

Table 1. Distribution of baseline clinical and laboratory characteristics by bloodstream infections groups.

	BSI	Non-BSI	Normal Control	Chi-square	*p*
	N = 16	N = 32	N = 20		
Sex = male (n, %)	10 (62.5%)	11 (34.4%)	10 (50%)	3.624	0.163
Age (years), median (IQR)	12.5 (5.7–14)	11 (7–14)	10.5 (7.3–12.8)		0.259
BMI, median (IQR)	16.2 (14.6–19.6)	16.3 (15.1–18.6)			0.759
Disease				3.295	0.193
AML	12	17			
ALL	3	6			
Others	1	9			
HCT-CI, median (IQR)	1 (1–1)	1 (1–1)			0.885
Disease risk				1.596	0.207
Standard	2	11			
High	14	21			
Disease state				0.381	0.537
Remission	6	15			
Unremission	10	17			
Donor					0.333
Non-related	1	0			
Related	15	32			
HLA-matching				6.077	0.049
10/10 matched	0	6			
6–9/10 matched	5	3			
5/10 matched	11	23			
Positive BSI history (n, %)	4 (25%)	5 (15.6%)		0.154	0.695
Conditioning regimen					
Myeloablative conditioning with BuCy	4 (25%)	10 (31.3%)		0.202	0.653
Total Body Irradiation	4 (25%)	2 (6.3%)		1.929	0.165
Post-Transplant Cyclophosphamide	8 (50%)	20 (62.5%)		0.686	0.408
Antibiotic use pre-transplant					
β-lactam	12	19		1.139	0.286
Aminoglycoside	6	9		0.436	0.509
Quinolones	1	1		0.000	1.000
Polypeptide	5	1		5.357	0.021
Macrolide	2	3		0.000	1.000
Carbapenem	13	20		1.745	0.186
Oxazolidinone	2	4		0.000	1.000
Vancomycin	8	8		3.000	0.083
Tigecycline	4	5		0.154	0.695
Fungicide	14	19		3.927	0.048
Antibiotic use post-transplant					
β-lactam	14	20		2.130	0.144
Aminoglycoside	11	17		1.071	0.301
Quinolones	1	2		0.000	1.000
Polypeptide	4	1		3.377	0.066
Macrolide	1	1		0.000	1.000
Carbapenem	14	26		0.019	0.891
Oxazolidinone	0	2			0.546
Vancomycin	11	20		0.182	0.670
Tigecycline	10	15		1.043	0.307
Fungicide	11	22		0.000	1.000
Vitamin D3 (125 IU/day) (n, %)	7 (43.75%)	7 (21.88%)		2.471	0.116

Table 1. Continued.

	BSI	Non-BSI	Normal Control	Chi-square	*p*
	N = 16	N = 32	N = 20		
Calcitriol (0.25 ug/day) (n, %)	3 (18.75%)	6 (18.75%)		0.000	1.000
Diarrhea pre-transplant (positive, %)	13 (81.2%)	20 (62.5%)		1.745	0.186
Diarrhea post-transplant (positive, %)	16 (100%)	26 (81.25%)		1.929	0.165
Agranulocytosis period (days), median (IQR)	32 (25–36)	24 (19–33)			0.126
Neutrophils, percent, median (IQR)	76.3 (64.5–86.7)	75 (60.5–83.8)			0.918
Hemoglobin (g/L), median (IQR)	82 (76–95)	84 (79–90)			0.918
Total protein (g/L), median (IQR)	61.7 (53.5–66.5)	56.5 (52.6–64.4)			0.126
Albumin (g/L), median (IQR)	38.9 (34.2–41.7)	35.8 (34.1–38.8)			0.358
Lactate dehydrogenase median (IQR)	315.5 (255.8–452.5)	283.5 (236.6–473.8)			0.759
Outcome (survival, %)	10 (62.5%)	28 (87.5%)		2.668	0.102

Abbreviations: IQR, Interquartile Range; BMI, denotes body mass index; AML, Acute Myeloid Leukemia; ALL, Acute Lymphocytic Leukemia; HCT-CI, hematopoietic cell transplantation specific comorbidity index; BSI, bloodstream infections; HLA, human leucocyte antigen; BuCy, Busulfan and Cyclophosphamide.

taxonomic units (OTUs) were obtained by classifying the same or similar sequences into the same feature. Alpha-diversity (α-diversity) was evaluated using three different metrics: richness (chao1), Shannon and OTUs (observed species). Weighted and unweighted UniFrac and Bray-Curtis distances were used to construct principal coordinates analysis (PCoA) graphs. The unweighted pair group method with arithmetic mean algorithm (UPGMA) was used to construct the tree structure. A parametric ANOVA test with a threshold of $p < 0.05$ was used to compare the GM taxonomic composition between groups. The significantly different species identified by this step were then analyzed with the Wilcoxon rank-sum test using a threshold of 0.05. Linear discriminant analysis (LDA) was subsequently performed to reduce the dimensionality of the data and to assess the influence of the significantly different species with a threshold set at 3. Multiple groups were compared by the Kruskal-Wallis statistical test, while the *t*-test was used to compare two groups. Anosim similarity analysis was also carried out. The gene function spectrum of different species was inferred using PICRUSt 2 software (https://github.com/picrust/picrust2/releases).

2.6 Statistical Methods

SPSS 22.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis of the clinical data. The data for discrete variables was presented as numbers and proportions, while data for continuous variables was presented as the median and range. Differences in patient characteristics between groups were assessed using the Mann-Whitney U test for continuous variables, and the chi-square test for categoric values. The Kruskal-Wallis H test was used to compare VD profiles between groups. Spearman analysis was used to analyze the correlation between VD levels and the abundance of the microbial community. To analyze the effect of risk factors on the incidence of BSI, relative risks were calculated using the crosstab method. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1 General Characteristics of the Study Participants

A total of 48 children who underwent HSCT were enrolled in the BSI and non-BSI patient groups. Sixteen (33.3%) patients suffered BSI during the period of agranulocytosis, and 32 (66.7%) did not. The distribution of baseline clinical characteristics and laboratory test results is shown in Table 1. Patient and control groups were not significantly different with respect to age and gender. No differences were found between the BSI and non-BSI patient groups with respect to age, sex, BMI, primary diagnosis, disease status, complete blood count, biochemical results, VD use and outcome, but significant differences were found for human leucocyte antigen (HLA) matching, as well as for polypeptide and fungicide use pre-transplantation. The mortality rate was 37.5% in the BSI group and 12.5% in the non-BSI group.

3.2 Serum Vitamin D Analysis

Of the 16 BSI patients, 7 (43.75%) received small dose oral supplementation with vitamin D3 (125 IU/day), and 3 (18.75%) received oral supplementation with calcitriol (0.25 µg/day). Of the 32 non-BSI patients, 7 (21.88%) received small dose oral supplementation with vitamin D3 (125 IU/day), and 6 (18.75%) received oral supplementation with calcitriol (0.25 µg/day). No significant differences in the levels of serum 25(OH)D and $1,25(OH)_2D_3$ were observed between patients who did and did not take VD, nor in the abundance of the GM ($p > 0.05$). Serum VD levels in the patient and healthy control groups are shown in Table 2. No significant differences in serum 25(OH)D and

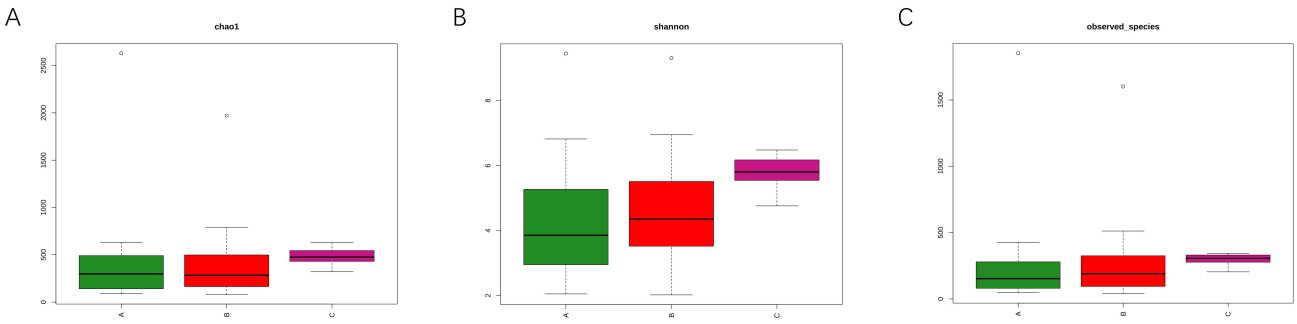

Fig. 1. Box plots of GM α-diversity comparisons among BSI patients (A), non-BSI patients (B) and healthy control groups (C). GM, gut microbiota.

Table 2. Status of Serum Vitamin D by bloodstream infections groups.

	BSI	Non-BSI	Normal Control	*p*
	N = 16	N = 32	N = 20	
25(OH)D pretransplant (ng/mL), median (IQR)	62.85 (39.79–86.46)	41.59 (32.22–63.19)	50.95 (41.56–63.05)	0.829
$1,25(OH)_2D_3$ pretransplant (pg/mL), median (IQR)	0.85 (0.6–3.55)	0.8 (0.6–2.87)		0.758
25(OH)D Posttransplant (ng/mL), median (IQR)	54.73 (40.75–78.88)	49.29 (40.67–64.55)		0.609
$1,25(OH)_2D_3$ Posttransplant (pg/mL) median (IQR)	0.75 (0.55–2.38)	1.72 (0.8–6.32)		0.759

25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D3.

$1,25(OH)_2D_3$ levels were found between the BSI and non-BSI groups before and after transplantation, nor in comparison to the normal control group. Moreover, no correlations were observed between 25(OH)D or $1,25(OH)_2D_3$ levels and patient outcome ($p > 0.05$).

3.3 Microbiota Analysis

3.3.1 α-Diversity

The abundance of fecal microbiota in BSI and non-BSI patients was significantly lower than in healthy subjects. The median value for Chao 1 in BSI (297.95, 130.52–504.04) and non-BSI (283.59, 152.92–514.75) patients was significantly lower ($p = 0.01$) than in normal controls (474.82, 430.18–558.66). The median value for Shannon was 3.85 (2.94–5.28), 4.35 (3.49–5.62) and 5.80 (5.51–6.20) in BSI, non-BSI and healthy controls, respectively, while for observed species the median was152.95 (78.75–293.75), 190 (89.25–335.22), and 305.95 (274.5–331.0), respectively ($p < 0.01$) (Fig. 1). No significant difference was found between the BSI and non-BSI patient groups ($p = 0.86$), although the richness of GM was slightly lower in BSI patients. No significant correlations were found between 25(OH)D or $1,25(OH)_2D_3$ levels and the abundance of flora ($p > 0.05$).

3.3.2 Taxonomic Composition

Characterization of the fecal microbial community is shown in Fig. 2. At the level of phylum, the BSI group had a significantly lower relative abundance of Firmicutes compared to the control group ($p = 0.009$), but not to the non-BSI group ($p = 0.08$). The abundance of Actinobacteria was not significantly different between the two patient groups ($p = 0.188$), but in both groups it was significantly less than in the control group ($p < 0.001$). The BSI group showed a significantly higher abundance of Proteobacteria compared to the non-BSI group ($p = 0.0434$), while the latter group had a significantly higher abundance than healthy controls ($p = 0.0193$). No significant difference in the abundance of Bacteroidetes was observed between the three groups ($p = 0.375$). At the genus level, the abundance of *Staphylococcus* ($p < 0.001$), *Pseudomonas* ($p < 0.001$), *Lactobacillus* ($p = 0.04966$), *Enterococcus* ($p < 0.001$), *Veillonella* ($p = 0.0172$), *Erysipelothrix* ($p = 0.0122$), *Clostridium innocuum* ($p = 0.0175$) and *Enterobacter* ($p = 0.0394$) were all significantly higher in the BSI and non-BSI patient groups compared to healthy subjects. *Clostridium innocuum* ($p = 0.0287$) was significantly higher in non-BSI patients compared to BSI patients. On the other hand, the abundance of *Bifidobacterium* ($p < 0.001$), *Faecalibacterium* ($p < 0.001$), *Agathobacter* ($p < 0.001$) and *Blautia* ($p < 0.001$) were each significantly lower in the patient groups compared to healthy subjects.

Fig. 3 shows a comparison of the fecal microbial community between BSI (Fig. 3A) and non-BSI (Fig. 3B) patients. The abundance of *Erysipelothrix* ($p = 0.0187$) and of *Clostridium innocuum* ($p = 0.0287$) was significantly lower in the BSI group compared to the non-BSI group.

LDA analysis was performed to identify species that were significantly different in abundance between subgroups (see **Supplementary Figure**). Characteristic flora in the healthy control group were *Bifidobacterium*, *Coriobacteriaceae*, *Lachnospiraceae*, *Butyricicoccaceae*, Os-

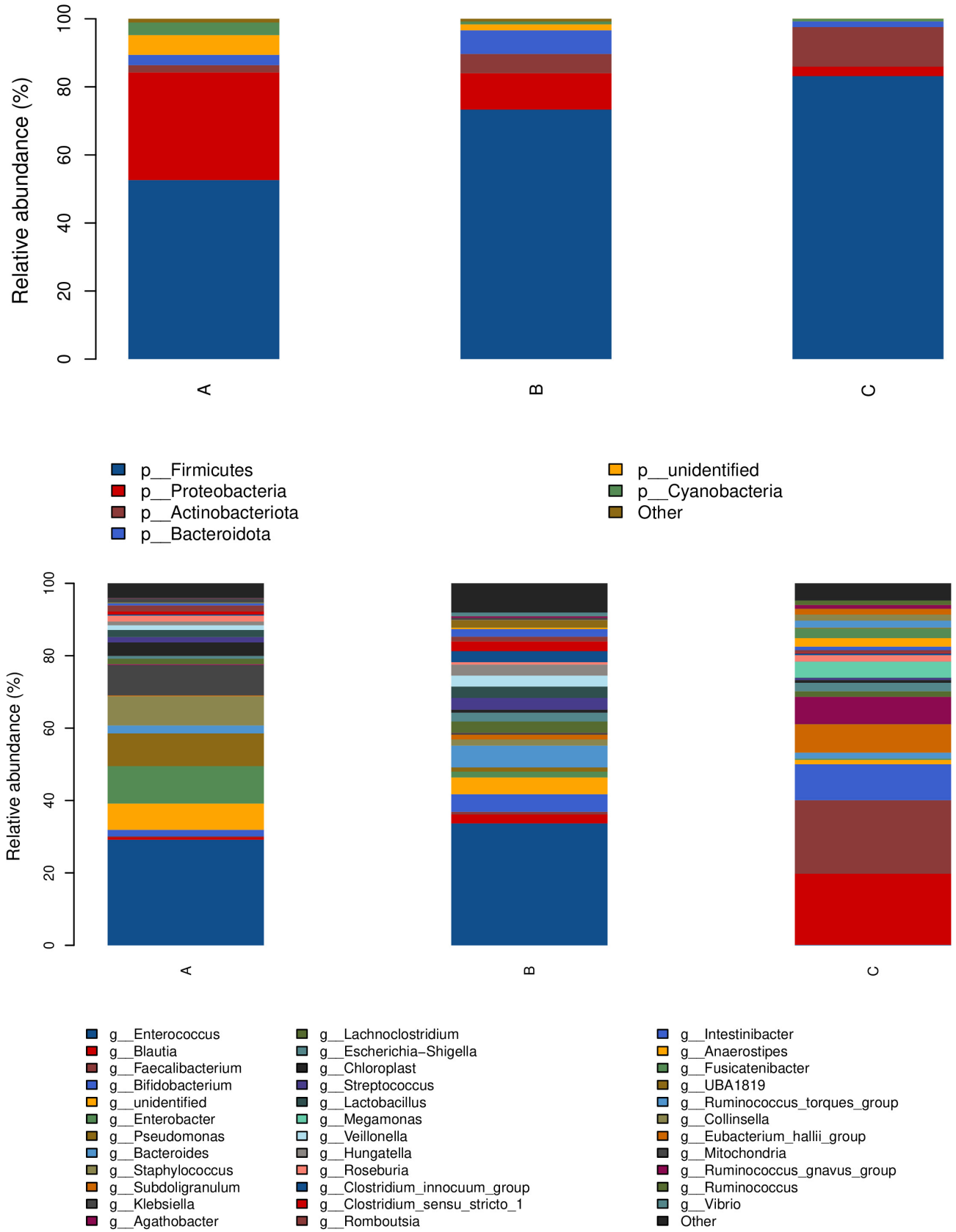

Fig. 2. Bar chart of fecal microbial community characterization in BSI (A), non-BSI (B) and healthy subjects (C) at the Phylum (p) and genus (g) levels.

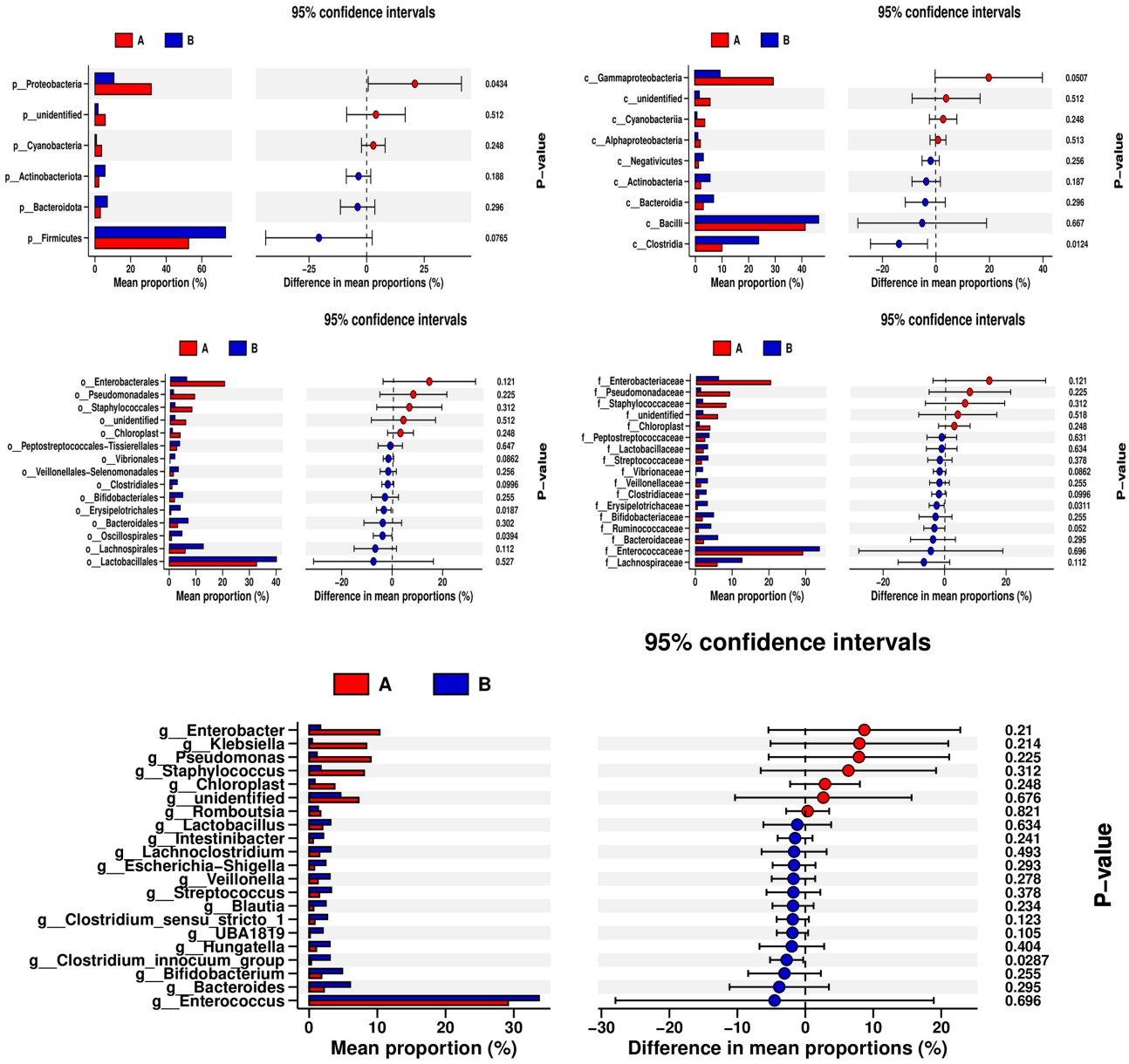

Fig. 3. Compared fecal microbial community characterization between BSI (A) and non-BSI (B) patients at the Phylum (p), class (c), order (o), family (f) and genus (g) levels.

cillospirales and *Selenomonadaceae*. In the non-BSI group they were *Actinomycetaceae*, *Erysipelotrichaceae*, *Enterococcaceae* and *Lactobacillaceae*, while in the BSI group they were *Staphylococcaceae*, *Enterobacteriaceae* and *Pseudomonadaceae*.

3.3.3 β-Diversity

The results of PCoA and UPGMA analysis are shown in Fig. 4. The distance ranges for microbiota in the BSI and non-BSI patients overlapped, and both were distant from the control group. UPGMA analysis also revealed a significant population difference between the patient and control groups. Anosim similarity analysis found there were significant differences between the three groups. The R statistic and p-value between BSI and non-BSI, BSI and control, and non-BSI and control groups were 0.1256 and 0.027, 0.6186 and 0.001, and 0.5487 and 0.001, respectively.

3.4 Clinical Analysis

3.4.1 Risk Factors for BSI and Death

The results of relative risk (RR) analysis showed that remission of the primary disease before transplantation may be apotential protective factor against BSI (RR = 0.709, 95% CI: 0.459–1.096). Potential risk factors for BSI were a positive history of BSI during chemotherapy (RR = 1.605, 95% CI: 0.816–3.157), gastric tube placement (RR = 3.000, 95% CI: 0.891–10.096), and diarrhea before transplantation (RR = 1.32, 95% CI: 0.908–1.918). Remission of

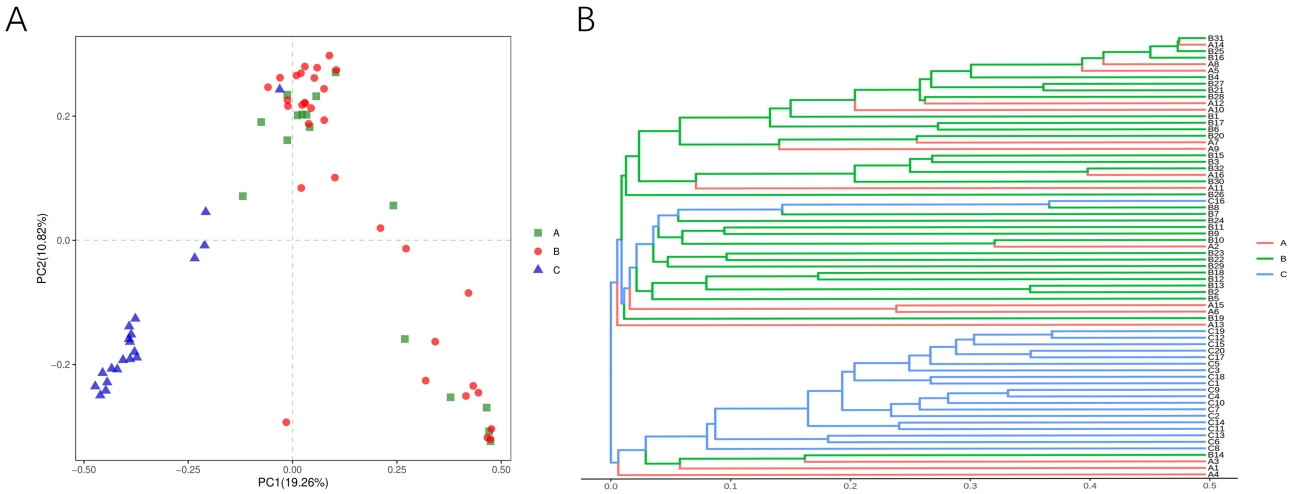

Fig. 4. PCoA and UPGMA analysis based on β-diversity of GM in different groups. (A) PCoA analysis based on β-diversity in BSI (green), non-BSI (red) and healthy (blue)groups. (B) UPGMA analysis based on β-diversity in BSI (red), non-BSI (green) and healthy (blue) groups. PCoA, construct principal coordinates analysis; UPGMA, unweighted pair group method with arithmetic mean algorithm; GM, gut microbiota; BSI, bloodstream infections.

the primary disease before transplantation was predictive of a better survival outcome (RR = 0.615, 95% CI: 0.43–0.877), whereas gastric tube placement (RR = 3.600, 95% CI: 1.079–12.008) and diarrhea after transplantation (RR = 1.313, 95% CI: 1.108–1.554) were risk factors for worse survival outcome.

3.4.2 Clinical Characteristics of Patients with BSI

The main clinical features, pathogens, and dominant GM of the 16 patients with BSI are shown in Table 3. The most common suspected source of infection was the intestine (10/16, 62.5%), followed by peripherally inserted central catheters (3/16, 18.8%) and skin lesions (2/16, 12.5%). A majority of patients (81.25%, 13/16) had diarrhea before the onset of BSI. Of the 10 suspected cases with pathogenic bacteria of intestinal origin, 3 (30%) had *Enterococcus*, 3 (30%) had *Klebsiella*, 3 (30%) had *Escherichia coli*, and 1 (10%) had *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The predominant intestinal flora of 7 (43.75%) patients was consistent with the pathogenic bacteria of BSI.

4. Discussion

VD plays a crucial role in innate immunity, adaptive immunity, endothelial function at the mucosal barrier, and the GM, while the GM is essential for host immune response and vitamin synthesis [4]. Combined research on VD and the intestinal microflora has led to significant progress in many inflammatory diseases, metabolic diseases, and tumors. However, the consequences and mechanism of VD deficiency and GM dysbiosis for BSI remain controversial [7,8,22,23], especially in young HSCT patients [24,25]. This study focused on VD and gut flora in pediatric HSCT patients in order to expand current knowledge in this evolving area.

There may be several reasons for the inconsistent conclusions in the literature regarding VD in pediatric HSCT patients. These include different levels of sunlight exposure in patients from different areas, different detection methods (ELISA, chemiluminescence, mass spectrometry) for the storage (25(OH)D) and active ($1,25(OH)_2D_3$) forms of VD, the timing of VD measurement, non-uniform diagnostic criteria for severe VD deficiency, and the relatively small sample size of pediatric HSCT studies. In the present work, we simultaneously detected serum 25(OH)D and $1,25(OH)_2D_3$ both pre- and post-transplantation. However, neither of these VD forms was found to be different between the BSI, non-BSI, and healthy control groups. Some patients had received low oral doses of VD or calcitriol for the prevention of osteoporosis associated with glucocorticoid use. Despite this, no significant differences were observed between the patient groups, possibly due to the much lower doses taken relative to the therapeutic doses used for VD deficiency [26]. Mechanistic study has demonstrated the involvement of VD in regulating the tight junction of the intestinal mucosal barrier, intestinal inflammatory cells and cytokines, and the abundance and composition of the GM [27]. However, the present study found no correlation between VD levels and the onset of BSI, patient outcome, or the diversity of intestinal flora. VD deficiency can decrease the diversity of the microbial community, with less Firmicutes and more Proteobacteria [28]. Moreover, VD supplementation increases the abundance of Firmicutes phylum [13]. The shifts in flora diversity and composition observed here in BSI patients compared to healthy children are similar to those reported previously in child and adolescent Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leuk-

Table 3. The main clinical signs, pathogens and dominant gut microbiota of patients with BSI.

Patient ID	Body temperature	Diarrhea pre-BSI	Low blood pressure	Oliguria	BSI pathogenic bacteria	Possible source of infection	Dominant bacterial community (Relative abundance >15%, *Genus*)	Outcome
A1	39.5	positive			*Staphylococcus epidermidis*	PICC	*Pseudomonas*, *Shinella*	death
A2	38.2				*Staphylococcus hominis*	PICC	*Clostridium*, *Bifidobacterium*, *Veillonella*	survival
A3	40	positive			*Enterococcus faecalis*	Intestinal	*Lactobacillus*	death
A4	39.9	positive	positive		*Escherichia coli*	Intestinal	Firmictues	death
A5	40	positive	positive		*Staphylococcus aureus*	Unspecified	*Enterococcus*, *Staphylococcus*	survival
A6	39	positive			*Deficient anaerobic bacteria*	Unspecified	*Klebsiella*, Gammaproteobacteria	survival
A7	39.8	positive	positive		*Staphylococcus epidermidis*	Unspecified	*Staphylococcus*, *Enterococcus*, *Enterobacter*	survival
A8	38.4	positive			*Pseudomonas aeruginosa* *Staphylococcus epidermidis*	PICC and Intestinal	*Enterococcus, Bacillus, Lactobacillus*	survival
A9	38.6	positive			*Fusarium*	Skin lesion of scrotum	*Staphylococcus, Klebsiella*	survival
A10	39.5	positive			*Enterococcus cecorum*	Intestinal	*Enterococcus, Klebsiella, Bacteroides*	death
A11	39.2	positive			*Klebsiella pneumoniae*	Intestinal	*Pseudomonas, Porphyromonas*	survival
A12	40.2				*Escherichia coli*	Intestinal	*Enterococcus, Roseburia, Escherichia*	death
A13	39	positive			*Klebsiella pneumoniae*	Intestinal and Perianal lesions	*Klebsiella, Enterococcus*	death
A14	41	positive	positive	positive	*Klebsiella pneumoniae*	Intestinal	*Enterococcus, Staphylococcus*	survival
A15	37.8	positive			*Enterococcus faecium* and *Staphylococcus haemolyticus*	Intestinal	*Klebsiella, Romboutsia, Staphylococcus*	survival
A16	39.2				*Escherichia coli*	Intestinal	*Enterococcus, Bacteroides, Enterobacter*	survival

PICC, peripherally inserted central catheters.

emia (AML) patients pre-chemotherapy and pre-transplantation [29,30]. An appropriately sized, randomized controlled trial using a specified dose of VD3 may allow a more definite conclusion to be reached. However, the current study did not find any practical value in monitoring VD levels in HSCT pediatric patients.

At the phylum level, the results of the present study concur with some other reports that also showed less Firmicutes, less or unchanged Actinobacteria, and more or unchanged Bacteroidetes between patients and healthy controls [29]. However, some discordant results were also found, as no difference was reported for Proteobacteria, whereas our study found significant differences between the three groups. This may be a result of transplantation and its complications. Conditioning regimens such as cytotoxic drugs, DNA-alkylating agents, radiotherapy, and the prophylactic use of broad-spectrum antibiotics result in major changes in microbiota composition. In particular, patients receiving radiotherapy were characterized by a relatively high abundance of Proteobacteria [31]. Proteobacteria showed the highest relative diversity in the BSI group, possibly because many pathogenic bacteria such as *Enterobacter*, *Escherichia* and *Pseudomonas* belong to this phylum. Taur *et al*. [32] found that the risk of gram-negative bacteremia in HSCT patients was 5-fold higher if the GM was dominated by Proteobacteria. The increased use of antibiotics for BSI can further exacerbate imbalances in the GM composition. Antibiotics often eradicate Bacteroidetes, Firmicutes and Actinobacteria taxa, thus opening a niche for colonization or proliferation by opportunistic bacteria from the Proteobacteria phylum [33].

Research over the past few years has indicated that sepsis and its treatment with antibiotics can lead to dysregulation of the microbiome. The gut has also been hypothesized to be the "motor" of sepsis [34]. Sepsis and intestinal dysbiosis interact in a vicious cycle, with sepsis inducing gut dysbiosis through various effects on gut hypoperfusion, immune dysregulation and organ failure. Moreover, sepsis can be induced through pathogen-associated molecular patterns (PAMPs), inflammatory cytokines, immune cell apoptosis, as well as injury to the intestinal epithelium [35] and tight junctions leading to a leaky gut and bacterial translocation [36]. Firmicutes are the main bacterial producers of SCFA, and reduced abundance leads to the invasion of pathogenic bacteria through the gut and into the blood circulation [35]. In the present study, a lower abundance of Firmicutes was observed in the patient groups. However, it was the predominant intestinal bacteria in almost half the BSI patients, consistent with previous reports on pathogenic bacteria in BSI. Although no data was available for GM composition prior to BSI, 62.5% of the pathogens in BSI may originate from the intestinal tract based on clinical observations, and most patients have diarrhea before the onset of BSI.

At the genus level, a consistent finding amongst studies including the current one is that *Faecalibacterium* is reduced in patients with acute leukemia [37,38]. The SCFA butyrate is produced mainly by *Faecalibacterium* and could be involved in intestinal barrier repair, as well as impeding the progression of AML [38]. Treatment with cefazolin, ampicillin, moxifloxacin, and vancomycin has been shown to decrease the *Faecalibacterium* population in humans [39]. In pediatric leukemia patients, *Bacteroides*, *Acinetobacter*, *Enterococcus* and *Veillonella* were reported to be significantly enriched, whereas *Bifidobacterium*, *Anaerostipes*, *Erysipelothrix*, *Faecalibacterium*, *Lactobacillus*, *Roseburia* and *Ruminococcus* were markedly reduced [40,41]. During HSCT, the intestinal flora may also suffer from the loss of probiotics such as *Faecalibacterium*, *Bifidobacterium* and *Ruminococcus*, and from colonization by non-probiotics such as *Enterococcus*, *Staphylococcus*, *Escherichia* and *Enterobacter* [42,43]. A high abundance of potential pathogens such as *Enterococcus* [44], *Staphylococcus* and *Pseudomonas* was also observed after BSI [23]. The present study found that Enterobacteriaceae was the characteristic flora of the BSI group. Treatment with Ampicillin, Amoxicillin/clavulanic acid and Clarithromycin/metronidazole has been shown to increase the *Enterobacteria* population [39].

5. Conclusions

In summary, this study found that measuring VD levels has no practical value in HSCT patients. Additionally, during HSCT and BSI, there was a loss of probiotics and an increase in potential pathogens in the gut microbiota.

The strengths of this study were its prospective study design, quality-controlled laboratory testing, and an experienced clinician. Limitations of the study included its single-center design which may have led to patient bias, the lack of fecal samples and hence GM data prior to transplantation and BSI onset, the lack of parallel testing of GM metabolites, and the relatively small number of pediatric HSCT patients. To corroborate our findings, further multi-center studies are required that have a large sample size, multiple time points for the testing of intestinal flora and metabolites, and additional monitoring indexes that reflect the actual VD state.

Availability of Data and Materials

The authors confirm that the data supporting the findings of this study are available within the article.

Author Contributions

QG and WJZ—designed the research, data collection, study supervision and final draft editing; MJB, TQQ, JZ and YS—performed the research and data analysis; QG—review of literature, performed the statistical analysis and drafted the manuscript. GWL—guided the design of the

study and provided help on the methodology of data analysis and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Aerospace Center Hospital (approval number: 2022065). All parents consented for the main trial to store these children's blood and fecal specimen for use, but not consented for future research.

Acknowledgment

We thank our patients, research and medical staff for making this study possible.

Funding

This research received funding from Aerospace Center Hospital Youth Innovation Fund (grant number 2022QN07).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJVNR26126.

References

[1] Yang M, Xin L, Li H, Lu X, Pan X, Lei S, *et al*. Risk factors for bloodstream infection in paediatric haematopoietic stem cell transplantation: a systematic review and meta-analysis. The Journal of Hospital Infection. 2023; 139: 11–22. https://doi.org/10.1016/j.jhin.2023.06.003.

[2] Lehrnbecher T, Averbuch D, Castagnola E, Cesaro S, Ammann RA, Garcia-Vidal C, *et al*. 8th European Conference on Infections in Leukaemia: 2020 guidelines for the use of antibiotics in paediatric patients with cancer or post-haematopoietic cell transplantation. The Lancet. Oncology. 2021; 22: e270–e280. https://doi.org/10.1016/S1470-2045(20)30725-7.

[3] Sahin U, Toprak SK, Atilla PA, Atilla E, Demirer T. An overview of infectious complications after allogeneic hematopoietic stem cell transplantation. Journal of Infection and Chemotherapy: Official Journal of the Japan Society of Chemotherapy. 2016; 22: 505–514. https://doi.org/10.1016/j.jiac.2016.05.006.

[4] Malaguarnera L. Vitamin D and microbiota: Two sides of the same coin in the immunomodulatory aspects. International Immunopharmacology. 2020; 79: 106112. https://doi.org/10.1016/j.intimp.2019.106112.

[5] Kroner JDC, Sommer A, Fabri M. Vitamin D every day to keep the infection away? Nutrients. 2015; 7: 4170–4188. https://doi.org/10.3390/nu7064170.

[6] Belsky JB, Wira CR, Jacob V, Sather JE, Lee PJ. A review of micronutrients in sepsis: the role of thiamine, l-carnitine, vitamin C, selenium and vitamin D. Nutrition Research Reviews. 2018; 31: 281–290. https://doi.org/10.1017/S0954422418000124.

[7] de Haan K, Groeneveld ABJ, de Geus HRH, Egal M, Struijs A. Vitamin D deficiency as a risk factor for infection, sepsis and mortality in the critically ill: systematic review and meta-analysis. Critical Care (London, England). 2014; 18: 660. https://doi.org/10.1186/s13054-014-0660-4.

[8] Xiao D, Zhang X, Ying J, Zhou Y, Li X, Mu D, *et al*. Association between vitamin D status and sepsis in children: A meta-analysis of observational studies. Clinical Nutrition (Edinburgh, Scotland). 2020; 39: 1735–1741. https://doi.org/10.1016/j.clnu.2019.08.010.

[9] Wallace G, Jodele S, Howell J, Myers KC, Teusink A, Zhao X, *et al*. Vitamin D Deficiency and Survival in Children after Hematopoietic Stem Cell Transplant. Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation. 2015; 21: 1627–1631. https://doi.org/10.1016/j.bbmt.2015.06.009.

[10] Beebe K, Magee K, McNulty A, Stahlecker J, Salzberg D, Miller H, *et al*. Vitamin D deficiency and outcomes in pediatric hematopoietic stem cell transplantation. Pediatric Blood & Cancer. 2018; 65: 10.1002/pbc.26817. https://doi.org/10.1002/pbc.26817.

[11] Ponnarmeni S, Kumar Angurana S, Singhi S, Bansal A, Dayal D, Kaur R, *et al*. Vitamin D deficiency in critically ill children with sepsis. Paediatrics and International Child Health. 2016; 36: 15–21. https://doi.org/10.1179/2046905515Y.0000000042.

[12] Hansson MEA, Norlin AC, Omazic B, Wikström AC, Bergman P, Winiarski J, *et al*. Vitamin d levels affect outcome in pediatric hematopoietic stem cell transplantation. Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation. 2014; 20: 1537–1543. https://doi.org/10.1016/j.bbmt.2014.05.030.

[13] Tabassum A, Ali A, Zahedi FD, Ismail NAS. Immunomodulatory Role of Vitamin D on Gut Microbiome in Children. Biomedicines. 2023; 11: 1441. https://doi.org/10.3390/biomedicines11051441.

[14] Docampo MD, Auletta JJ, Jenq RR. Emerging Influence of the Intestinal Microbiota during Allogeneic Hematopoietic Cell Transplantation: Control the Gut and the Body Will Follow. Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation. 2015; 21: 1360–1366. https://doi.org/10.1016/j.bbmt.2015.02.016.

[15] Klingensmith NJ, Coopersmith CM. Gut Microbiome in Sepsis. Surgical Infections. 2023; 24: 250–257. https://doi.org/10.1089/sur.2022.420.

[16] Waterhouse M, Hope B, Krause L, Morrison M, Protani MM, Zakrzewski M, *et al*. Vitamin D and the gut microbiome: a systematic review of in vivo studies. European Journal of Nutrition. 2019; 58: 2895–2910. https://doi.org/10.1007/s00394-018-1842-7.

[17] Haak BW, Prescott HC, Wiersinga WJ. Therapeutic Potential of the Gut Microbiota in the Prevention and Treatment of Sepsis. Frontiers in Immunology. 2018; 9: 2042. https://doi.org/10.3389/fimmu.2018.02042.

[18] Morkis IVC, Vicente BDM, Habigzang M, da Silva PDO, Scherer F, Paz A, *et al*. Should we evaluate intestinal microbiota of pediatric patients undergoing hematopoietic stem cell transplantation? Bone Marrow Transplantation. 2020; 55: 1506–1508. https://doi.org/10.1038/s41409-019-0727-6.

[19] Zama D, Muratore E, Biagi E, Forchielli ML, Rondelli R, Candela M, *et al*. Enteral nutrition protects children undergoing

allogeneic hematopoietic stem cell transplantation from blood stream infections. Nutrition Journal. 2020; 19: 29. https://doi.org/10.1186/s12937-020-00537-9.

[20] Sankar J, Thakral V, Bharadwaj K, Agarwal S, Kabra SK, Lodha R, *et al*. The Microbiome and Metabolome of the Gut of Children with Sepsis and Septic Shock. Journal of Intensive Care Medicine. 2024; 39: 514–524. https://doi.org/10.1177/08850666231216361.

[21] Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatric Critical Care Medicine: a Journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies. 2005; 6: 2–8. https://doi.org/10.1097/01.PCC.0000149131.72248.E6.

[22] Delrue C, Speeckaert R, Delanghe JR, Speeckaert MM. Vitamin D Deficiency: An Underestimated Factor in Sepsis? International Journal of Molecular Sciences. 2023; 24: 2924. https://doi.org/10.3390/ijms24032924.

[23] Adelman MW, Woodworth MH, Langelier C, Busch LM, Kempker JA, Kraft CS, *et al*. The gut microbiome's role in the development, maintenance, and outcomes of sepsis. Critical Care (London, England). 2020; 24: 278. https://doi.org/10.1186/s13054-020-02989-1.

[24] Hong S, Ferraro CS, Hamilton BK, Majhail NS. To D or not to D: vitamin D in hematopoietic cell transplantation. Bone Marrow Transplantation. 2020; 55: 2060–2070. https://doi.org/10.1038/s41409-020-0904-7.

[25] Wang H, Zhong Y, Ma L. Leukaemia Infection Diagnosis and Intestinal Flora Disorder. Current Molecular Medicine. 2022; 22: 2–7. https://doi.org/10.2174/1566524021666210302144720.

[26] Holick MF. Vitamin D deficiency. The New England Journal of Medicine. 2007; 357: 266–281. https://doi.org/10.1056/NEJMra070553.

[27] Akimbekov NS, Digel I, Sherelkhan DK, Lutfor AB, Razzaque MS. Vitamin D and the Host-Gut Microbiome: A Brief Overview. Acta Histochemica et Cytochemica. 2020; 53: 33–42. https://doi.org/10.1267/ahc.20011.

[28] Cantorna MT, Snyder L, Arora J. Vitamin A and vitamin D regulate the microbial complexity, barrier function, and the mucosal immune responses to ensure intestinal homeostasis. Critical Reviews in Biochemistry and Molecular Biology. 2019; 54: 184–192. https://doi.org/10.1080/10409238.2019.1611734.

[29] Peppas I, Ford AM, Furness CL, Greaves MF. Gut microbiome immaturity and childhood acute lymphoblastic leukaemia. Nature Reviews. Cancer. 2023; 23: 565–576. https://doi.org/10.1038/s41568-023-00584-4.

[30] Guevara-Ramírez P, Cadena-Ullauri S, Paz-Cruz E, Tamayo-Trujillo R, Ruiz-Pozo VA, Zambrano AK. Role of the gut microbiota in hematologic cancer. Frontiers in Microbiology. 2023; 14: 1185787. https://doi.org/10.3389/fmicb.2023.1185787.

[31] Ciernikova S, Kasperova B, Drgona L, Smolkova B, Stevurkova V, Mego M. Targeting the gut microbiome: An emerging trend in hematopoietic stem cell transplantation. Blood Reviews. 2021; 48: 100790. https://doi.org/10.1016/j.blre.2020.100790.

[32] Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, *et al*. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America. 2012; 55: 905–914. https://doi.org/10.1093/cid/cis580.

[33] Fishbein SRS, Mahmud B, Dantas G. Antibiotic perturbations to the gut microbiome. Nature Reviews. Microbiology. 2023; 21: 772–788. https://doi.org/10.1038/s41579-023-00933-y.

[34] Miller WD, Keskey R, Alverdy JC. Sepsis and the Microbiome: A Vicious Cycle. The Journal of Infectious Diseases. 2021; 223: S264–S269. https://doi.org/10.1093/infdis/jiaa682.

[35] Chancharoenthana W, Kamolratanakul S, Schultz MJ, Leelahavanichkul A. The leaky gut and the gut microbiome in sepsis - targets in research and treatment. Clinical Science (London, England: 1979). 2023; 137: 645–662. https://doi.org/10.1042/CS20220777.

[36] Zuccaro V, Lombardi A, Asperges E, Sacchi P, Marone P, Gazzola A, *et al*. The Possible Role of Gut Microbiota and Microbial Translocation Profiling During Chemo-Free Treatment of Lymphoid Malignancies. International Journal of Molecular Sciences. 2019; 20: 1748. https://doi.org/10.3390/ijms20071748.

[37] Chua LL, Rajasuriar R, Azanan MS, Abdullah NK, Tang MS, Lee SC, *et al*. Reduced microbial diversity in adult survivors of childhood acute lymphoblastic leukemia and microbial associations with increased immune activation. Microbiome. 2017; 5: 35. https://doi.org/10.1186/s40168-017-0250-1.

[38] Wang R, Yang X, Liu J, Zhong F, Zhang C, Chen Y, *et al*. Gut microbiota regulates acute myeloid leukaemia via alteration of intestinal barrier function mediated by butyrate. Nature Communications. 2022; 13: 2522. https://doi.org/10.1038/s41467-022-30240-8.

[39] Ramakrishna BS, Patankar R. Antibiotic-associated Gut Dysbiosis. The Journal of the Association of Physicians of India. 2023; 71: 62–68. https://doi.org/10.59556/japi.71.0381.

[40] Liu X, Zou Y, Ruan M, Chang L, Chen X, Wang S, *et al*. Pediatric Acute Lymphoblastic Leukemia Patients Exhibit Distinctive Alterations in the Gut Microbiota. Frontiers in Cellular and Infection Microbiology. 2020; 10: 558799. https://doi.org/10.3389/fcimb.2020.558799.

[41] Gao X, Miao R, Zhu Y, Lin C, Yang X, Jia R, *et al*. A new insight into acute lymphoblastic leukemia in children: influences of changed intestinal microfloras. BMC Pediatrics. 2020; 20: 290. https://doi.org/10.1186/s12887-020-02192-9.

[42] Masetti R, Zama D, Leardini D, Muratore E, Turroni S, Prete A, *et al*. The gut microbiome in pediatric patients undergoing allogeneic hematopoietic stem cell transplantation. Pediatric Blood & Cancer. 2020; 67: e28711. https://doi.org/10.1002/pbc.28711.

[43] Uribe-Herranz M, Klein-González N, Rodríguez-Lobato LG, Juan M, de Larrea CF. Gut Microbiota Influence in Hematological Malignancies: From Genesis to Cure. International Journal of Molecular Sciences. 2021; 22: 1026. https://doi.org/10.3390/ijms22031026.

[44] Kang H, Thomas RM. Bacteria and Sepsis: Microbiome to the Rescue? Journal of Clinical Medicine. 2021; 10: 3578. https://doi.org/10.3390/jcm10163578.