

Letter to the Editor

Gut microbiome variations during hematopoietic stem cell transplant in severe combined immunodeficiency

To the Editor:

The composition of the gut microbiome is unstable in infancy,¹ with many environmental factors determining its development including mode of delivery, method of feeding, antibiotic exposure, and neonatal hospitalisation.² Severe combined immunodeficiency (SCID), a primary T-lymphocyte immunodeficiency causing severe viral infections, *Pneumocystis jirovecii* pneumonitis, chronic diarrhea, and failure to thrive, is fatal in infancy without hematopoietic stem cell transplantation (HSCT). Once diagnosed, children with SCID receive prolonged courses of antibiotics and breast-feeding frequently ceases during this critical time for gut microbiome development.³ Chemotherapeutic conditioning often precedes HSCT and can cause mucositis and severe immunosuppression, increasing the risk of bacteremia. HSCT can be complicated by graft-versus-host-disease (GvHD) causing severe gut inflammation. In HSCT in adults, overgrowth by a dominant gut microbiome species and the subsequent reduced diversity have been associated with gut GvHD^{4,5} and bacteremia.⁶ However, the gut microbiome in HSCT in infancy, specifically in SCID, has not been explored.

In this pilot study, we examined changes in the gut microbiome during HSCT for SCID. With informed parental consent, longitudinal daily stool samples were collected from 3 patients (see Table E1 in this article's Online Repository at www.jacionline.org) and analyzed on the basis of the occurrence of clinical events including onset of conditioning, HSCT, neutrophil engraftment, development of fever, bacteremia, or infection, antibiotic exposure, and GvHD. DNA was extracted from 81 stool samples by using the PowerLyzer PowerSoil kit (Mo Bio Laboratories, Carlsbad, Calif).⁷ 16S rDNA sequencing was performed on the MiSeq (Illumina, San Diego, Calif) platform, and raw data were processed in Mothur (University of Michigan)

following the Schloss laboratory standard operating procedure (http://www.mothur.org/wiki/MiSeq_SOP). Bacterial profiles were analyzed using partial least squares discriminant analysis (SIMCA, Umetrics, San Jose, Calif), and diversity was determined using the Shannon diversity index.

Partial least squares discriminant analysis (Fig 1) was performed on all samples, with grouping according to pre- or posttransplant status. This revealed 2 distinctive microbiome profile groups in relation to transplantation. One pretransplant sample (A_17_1) fell outside of the Hotellings T2 range, at 95% CI but had no significant clinical event associated with it. Two posttransplant samples fell outside of the Hotellings T2 range, at 95% CI. Sample A_17_2 was taken 1 week after starting teicoplanin and meropenem, the latter being switched for ceftazidime after 36 hours. Sample D_14_10 was taken after 5 days use of teicoplanin and meropenem and coincided with the diagnosis of disseminated cytomegalovirus infection and grade II GvHD.

Patient A was 34 weeks old on admission and 41 weeks old at HSCT. Shortly after stopping teicoplanin, meropenem, and treatment dose cotrimoxazole (pretransplant day -31), community diversity decreased (see Fig E1, Baby A, in this article's Online Repository at www.jacionline.org) as staphylococcal species dominated until after day -14 (Fig 2, Baby A). Staphylococcal species were dominant again from day -3 throughout the transplant period to day +36 posttransplant, despite multiple courses of broad-spectrum antibiotic and antifungal agents and neutrophil engraftment at day +19. Diversity during this period increased but never regained the diversity of early pretransplant samples. Between day +36 and +54, Shannon diversity remained low (<1). After stopping antibiotics and continuing antifungals, there was a change in the dominant taxa from staphylococci to enterococci.

Patient B was 6 days old on admission and 6 weeks at the time of conditioned transplant. Before transplant the microbiome appeared stable, with codominance of streptococcal species and

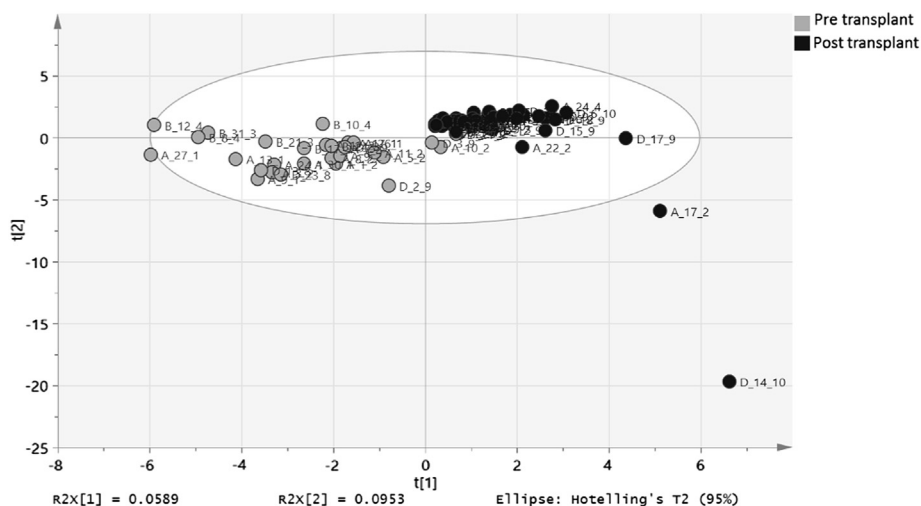
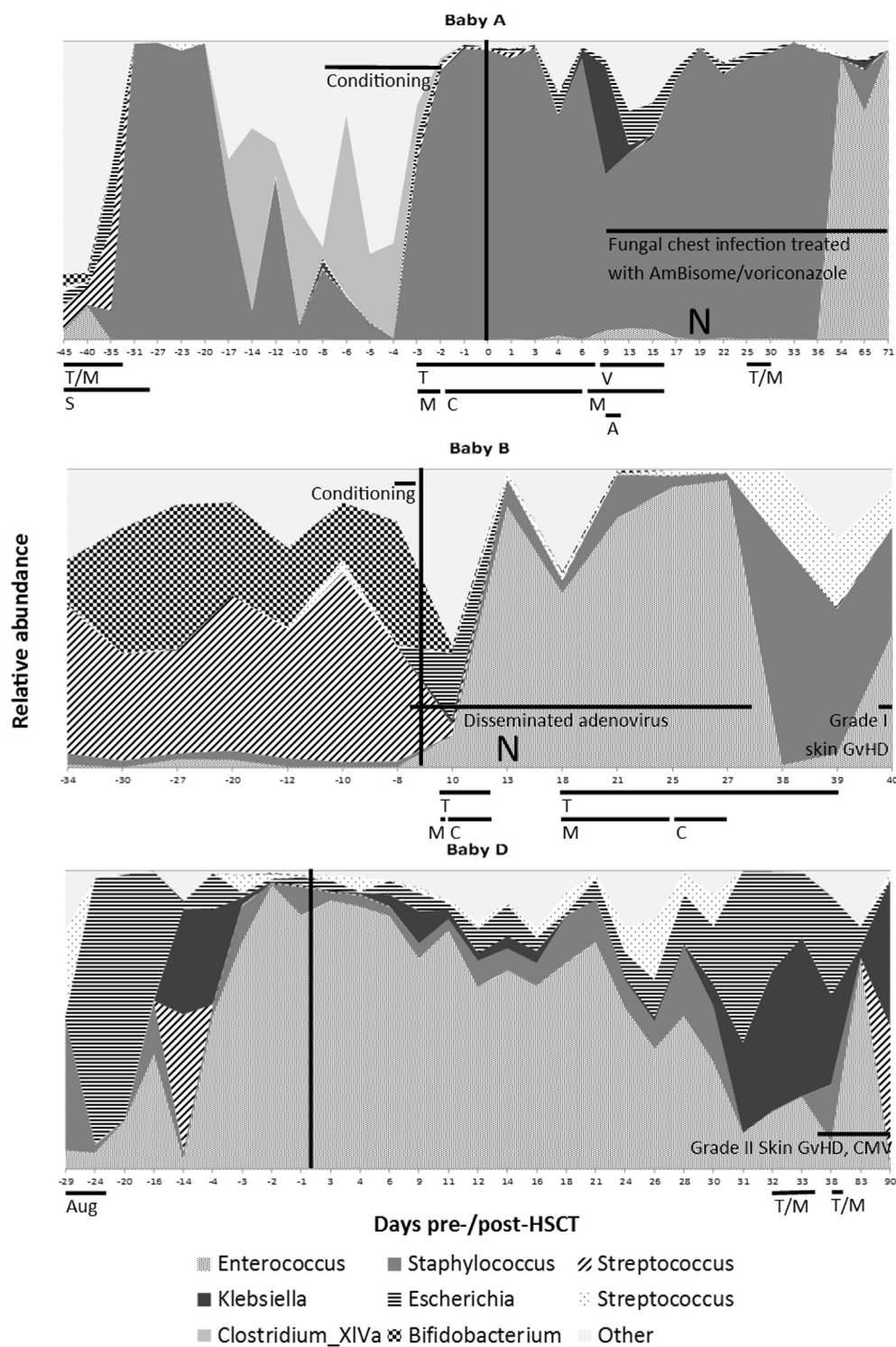


FIG 1. PLS-DA (partial least squares discriminant analysis) combining the gut microbiome profile of all 3 infants. Samples were designated as pre- or post-HSCT samples. The analysis shows that the samples can be grouped according to pre- and post-HSCT categories.



| Day 0, day of stem cell transplant

N Day of neutrophil engraftment

— Antibiotics listed below charts, line length indicates duration: T — teicoplanin; M — meropenem; V — vancomycin; S — co-trimoxazole; C — ceftazidime; A — amikacin; Aug — co-amoxiclav

FIG 2. Changes in proportional bacterial profile of the gut microbiome in babies A, B, and D over the period of HSCT. Significant events are noted including chemotherapy, day of HSCT (day 0), antibiotic use, significant infections, and GvHD occurrence.

bifidobacterium (Fig 2, Baby B) and a Shannon diversity of 1.2 to 1.6 (Fig E1, Baby B). Diversity decreased as enterococci dominated the community, from commencing conditioning until the point of neutrophil engraftment (day +13). This occurred alongside disseminated adenovirus infection. Diversity increased again only at the end of the sampling period. After day +38, there was codominance of enterococci, streptococci, and staphylococci.

Patient D was 5 days old on admission and 5 weeks at the time of unconditioned stem cell infusion. *Escherichia* species dominated initial samples but after day –14 this changed to a dominance of enterococci (Fig 2, Baby D), associated with a low diversity (Fig E1, Baby D). Shannon diversity increased after infusion and by day +21 the abundance of enterococci reduced in accordance with an increase in *Escherichia* species and *Klebsiella* species in the microbiome. Reemergence of staphylococci occurred in the days around the diagnosis of grade II skin GvHD and cytomegalovirus infection.

This pilot study is the first to describe the gut microbiome in SCID and demonstrates that the gut microbiome can be differentiated into pre- and post-HSCT groups. The small number of patients restricted detailed analysis; however, episodes of low diversity and dominance of varying species on an individualized basis around different HSCT events were evident.

Low diversity and bacterial dominance in gut microbiome associate with the development of bacteremia and gut GvHD in HSCT in adults.^{5,6} Enterococcal abundance of more than 20% has been associated with an increased frequency of gut GvHD in adults.⁴ In our study, patients B and D, diagnosed with SCID at birth, had enterococcal dominance during the initial posttransplant period but neither developed gut GvHD. Our population is not directly comparable to adults with HSCT because the infant gut microbiome is unstable over the first year of life.¹ Changes in the gut microbiome in infants and children with HSCT and its association with antibiotic use and gut GvHD warrant further work to complement adult studies.

We aimed to explore gut microbiome alterations in HSCT for SCID rather than compare SCID gut microbiome to “normal.” Development of the “healthy” infant gut microbiome has been described previously and shown to be individualized with a higher bacterial diversity than seen in our study.^{1,8} This, combined with significant antibiotic exposure associated with SCID and HSCT, means that comparison of pre- and post-HSCT samples is of more value than comparison with healthy age-matched controls.

During HSCT, antibiotics and conditioning are usually required and therefore cannot be altered to influence the

microbiome. Data on nutrition were collected, which show varied feeding methods (see Table E1). However, our study is unable to show associations between microbiome and feeding methods. The role of nutritional intervention is being studied in necrotizing enterocolitis and sepsis in neonates.^{7,9} Future work could explore the role of nutritional interventions in changes in the gut microbiome in HSCT for SCID and outcomes of GvHD and sepsis.

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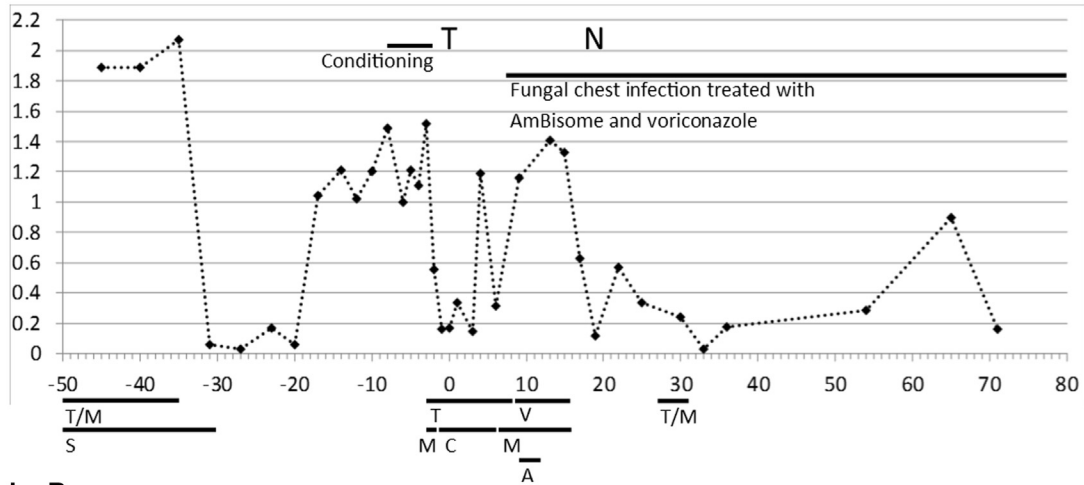
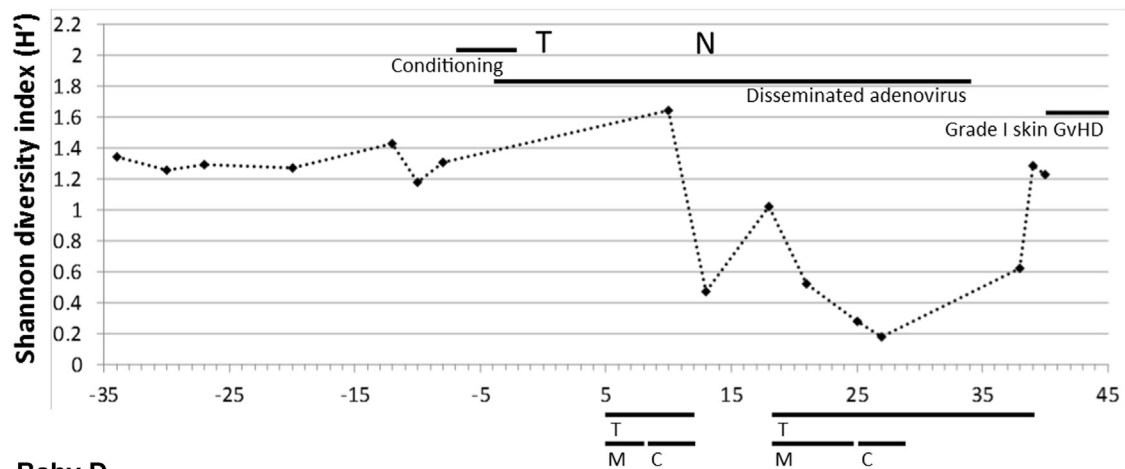
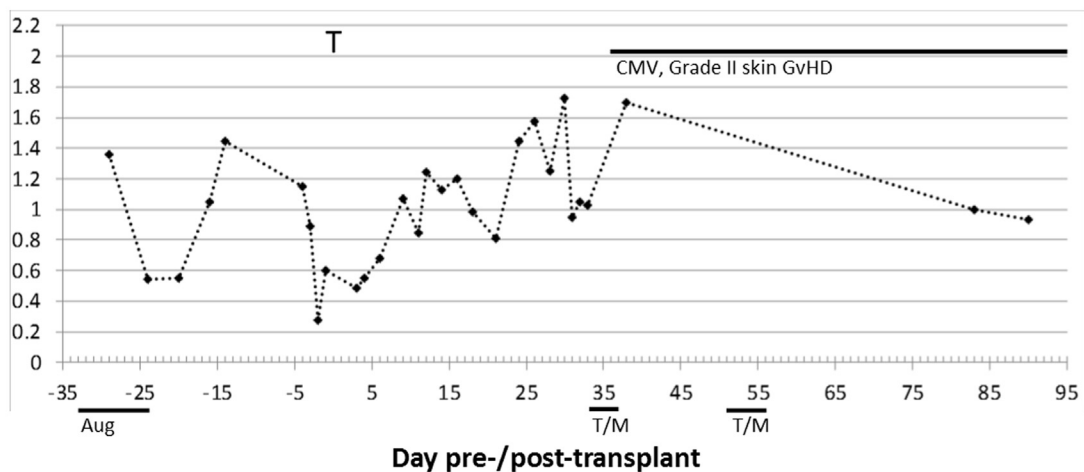
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Baby A**Baby B****Baby D**

T Day 0, day of stem cell transplant

N Day of neutrophil engraftment

— Events, line length indicates duration. Antibiotics: T — teicoplanin; M — meropenem; V — vancomycin; S — co-trimoxazole; C — ceftazidime; A — amikacin; Aug — co-amoxiclav

FIG E1. Changes in Shannon diversity index in babies A, B, and D over the period of HSCT. Significant events are noted including chemotherapy, day of HSCT (day 0), antibiotic use, significant infections, and GvHD occurrence.

TABLE E1. Patient characteristics

Patient	Diagnosis	Age at admission (wk)	Age at HSCT (wk)	Conditioning	Donor/source	Nutrition	Complications
Baby A	X-linked common gamma chain	34	41	Treosulphan, fludarabine, Campath	MUD/cord	NG enteral feeds to day +7; PN day +7 to +45, NG feeds reintroduced gradually from day +16	<i>Pneumocystis jiroveci</i> pneumonia pre-HSCT, slow neutrophil engraftment, fungal chest infection
Baby B	RAG I	<1	6	Treosulphan, fludarabine, Campath	MFD/PBSC	Breast-fed until day −36. then bottle feeds with NG top up feed day +5 to +26 and day +45 onward	Disseminated adenovirus, CVL infection, GvHD skin grade I
Baby D	X-linked common gamma chain	<1	5	Nil	MSD/BMT	Breast-fed throughout except for NG feeds day +39 to +42	GvHD skin grade II, CMV infection

BMT, Bone marrow transplant; *CMV*, cytomegalovirus; *CVL*, central venous line; *MFD*, matched family donor; *MSD*, matched sibling donor; *MUD*, matched unrelated donor; *NG*, nasogastric; *PBSC*, peripheral blood stem cell; *PN*, parenteral nutrition.