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MAJOR ARTICLE

Gut Microbiome Composition Predicts Infection Risk During Chemotherapy in Children With Acute Lymphoblastic Leukemia

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Background. Myelosuppression-related infections remain important causes of morbidity and mortality in children with acute lymphoblastic leukemia (ALL).

Methods. By analyzing fecal samples collected at diagnosis and after each of the initial 3 phases of chemotherapy, we evaluated the role of gut microbiota in predicting infections in 199 children with newly diagnosed ALL. The bacterial 16S rRNA gene was analyzed by high-depth sequencing to determine the diversity and composition of the microbiome.

Results. After the induction and reinduction I phases of chemotherapy, microbial diversity decreased significantly relative to the prechemotherapy value. After chemotherapy, the relative abundance of certain bacterial taxa (eg, Bacteroidetes) decreased significantly, whereas that of other taxa (eg, Clostridiaceae and Streptococcaceae) increased. A baseline gut microbiome characterized by Proteobacteria predicted febrile neutropenia. Adjusting for the chemotherapy phase and ALL risk level, Enterococcaceae dominance (relative abundance ≥30%) predicted significantly greater risk of subsequent febrile neutropenia and diarrheal illness, whereas Streptococcaceae dominance predicted significantly greater risk of subsequent diarrheal illness.

Conclusions. In children undergoing therapy for newly diagnosed ALL, the relative abundance of Proteobacteria before chemotherapy initiation predicts development of febrile neutropenia, and domination of the gut microbiota by Enterococcaceae or Streptococcaceae at any time during chemotherapy predicts infection in subsequent phases of chemotherapy.

Clinical Trial Registration. NCT00549848.

Keywords. microbiome; cancer; children; acute lymphoblastic leukemia; infection.

Emerging epidemiologic evidence shows associations between gut microbiota and overall health [1–3]. Alterations in gut microbiota affect the immune system and resistance to infection in healthy individuals, adults with acute myeloblastic leukemia or non-Hodgkin lymphoma, and hematopoietic stem cell transplant (HSCT) recipients [4–7]. Disruption of the gut microbiome by chemotherapy and broad-spectrum antibiotics may facilitate domination by a single pathogen that can cross the intestinal mucosa to the bloodstream [8, 9]. The diversity and composition of gut microbiome predict infection in adults with cancer [5, 6, 8–10]. Although one study [11] showed that children with cancer had less-diverse gut microbiota than their healthy siblings, the relevance of the microbiome to the outcome of antineoplastic therapy in children is unknown.

We hypothesized that changes in the gut microbiome are associated with infection risk in children with ALL. This study examined changes in fecal microbiota in children with ALL before and during chemotherapy and aimed to identify characteristics of gut microbiome that predicted outcomes such as diarrhea, bloodstream infections, or febrile neutropenia (FN).

METHODS

Study Design and Participants

Patients were enrolled at the time of ALL diagnosis at St Jude Children's Research Hospital between January 2012 and August 2015. The protocol was approved by the institutional review board, and informed consent was obtained. The ALL treatment regimen and risk classification (ClinicalTrials.gov identifier NCT00549848) were described previously [12, 13]. Therapy consisted of a 6-week remission induction, 8-week consolidation, and 120-week continuation phase that included two 3-week periods of intensive chemotherapy (reinduction I in weeks 7–9 and reinduction II in weeks 17–20). Demographic, antibiotic and

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probiotic exposure, treatment, and outcomes were abstracted from the study database and electronic medical records.

Bloodstream infection was defined as detection of bacteria or fungi in blood cultures obtained for clinical care; diarrhea as ≥3 loose or watery stools in a 24-hour period or a documented clinical diagnosis; fever as an oral temperature >38.0°C persisting for >1 hour; and neutropenia as an absolute neutrophil count ≤500 cells/μL. Patients were followed from the date of ALL diagnosis until censoring at the first of: end of reinduction II, death, or removal from the Total XVI study because of ALL relapse, HSCT, or other factors.

Sample Collection

Stool specimens were obtained at 4 predefined time points for microbiome analysis: before or within 72 hours after induction chemotherapy initiation (baseline); within 2 weeks after induction completion (postinduction); and at the initiation of reinduction I (postconsolidation) and reinduction II (postreinduction) phases. Samples were frozen at −80°C until DNA extraction.

DNA Extraction, Library Construction, and Sequence Analysis

DNA was extracted by bead beating on a FastPrep instrument (MP Biomedicals, Santa Ana, California) followed by genomic DNA extraction with a FastDNA kit (MP Biomedicals). The V1–V3 region of the bacterial 16S rRNA gene was amplified using a NEXTflex 16S Amplicon-Seq Library Prep kit (Bioo Scientific, Austin, Texas). The multiplexed products were used for high-depth sequencing on the Illumina Mi-seq platform. The quality of raw 16S ribosomal RNA (rRNA) pair-ended reads (300 bp ×2) was examined by FastQC, and low-quality read ends (quality score <20) were trimmed with Trim Galore [14, 15]. The reads were subsequently merged with PANDAseq and processed with QIIME [16, 17]. The average read depth is 133 290 (median, 147 411). The closed-reference mapping protocol of QIIME was used for operational taxonomic unit (OTU) assignment at 97% sequence similarity, using the UCLUST algorithm [18]. A representative sequence was selected from each OTU for taxonomic assignment with the Greengenes database [19]. Using the OTU table, alpha diversity estimates (Shannon, Simpson, and Chao 1 indices) were calculated using R phyloseq [20]. Rarefaction curves were generated for number of sequences per sample (Supplementary Figure 1).

All sequence read files are accessible through the National Center for Biotechnology Information (project accession number PRJNA449103).

Statistical Analysis

OTU Assignment

All samples were included in the analysis. OTUs were grouped in 14 taxa based on frequency (Supplementary Figure 2).

Changes in Microbiota Diversity and Composition During Chemotherapy

A linear mixed-effects model examined changes in the diversity indices using SAS version 9.4 software (SAS Institute, Cary, North Carolina). For microbiome compositional change, a mixed-effects negative binomial regression model was used [21]. The *P* value was adjusted for the false discovery rate with the Benjamini-Hochberg method [22] and reported as *Q* value.

Effect of Antibiotics and Probiotics on Microbiome

Antibiotic prophylaxis against pneumocystis pneumonia was excluded from analysis because of ubiquitous exposure. Because all patients received other antibiotics during the study, exposure was calculated as the proportion of days receiving antibiotics for each chemotherapy phase. Correlation between antibiotic exposure and change in diversity was evaluated using Spearman rank test, and change in dominance of Bacteroidetes was tested using Fisher exact test. Probiotic use was analyzed using a Swimmer plot.

Impact of Baseline Microbiota on Infection Outcomes

For initial risk stratification at the time of ALL diagnosis, the Wilcoxon-Mann-Whitney test and logistic regression were used to assess potential risk factors for subsequent infection occurring at any time during the study period, including the baseline microbiome. For each taxon whose relative abundance (RA) significantly differed among patients who had or did not have the event, the RA was dichotomized at the optimal cutoff value based on the Youden index. Factors with a *P* value of <.1 in univariate analysis were examined by multiple logistic regression. The cumulative incidence of outcomes was compared among risk factors with Gray test.

Microbiota Composition Type Assignment

The "community state" refers to the RA of taxa in a stool sample at a specific time point. The "microbiota composition type" is a cluster of community states with similar microbiota composition. The community states were grouped using hierarchical clustering of the Jensen-Shannon divergence matrix, which assesses beta diversity and the optimal number of clusters [23], and ward linkage and presented as a heat map [21].

Modeling Association Between Microbiota and Infection Outcomes

To evaluate the effect of the time-dependent microbiome on the recurrent infection outcomes throughout all courses of chemotherapy, Anderson-Gill proportional hazards models were adopted [24, 25]. ALL risk level, Shannon diversity index and microbiome composition at the collection time point, and the chemotherapy phase were treated as time-dependent predictors. To assess sensitivity of the results, a bootstrap method (with 10 000 replicates) was used to examine the association between Enterococcaceae or Streptococcaceae dominance and subsequent infections. Dominance was defined as an RA of ≥30% [9].

RESULTS

Patient Characteristics and Infection Outcomes

A total of 199 children provided 406 stool samples (Table 1). Twenty-six patients (13%) had 31 episodes of bloodstream infection, 122 (61%) had 248 FN episodes, and 73 (37%) had

Table 1. Patient Characteristics (N = 199)

Characteristic	No. (%)
Age at diagnosis, y, median (range)	6.4 (0.5–18.4)
Age at diagnosis, y, No. (%)	
<1.5	9 (5)
1.5–4	72 (36)
5–9	65 (33)
≥10	53 (27)
Sex, No. (%)	
Male	118 (59)
Female	81 (41)
Race, No. (%)	
Black	28 (14)
White	158 (79)
Other	13 (7)
Acute lymphoblastic leukemia risk level at induction	
Low	106 (53)
Standard	81 (41)
High	12 (6)
Stool samples, No. (%)	406
Baseline before induction	112 (28)
After induction completion	97 (24)
Postconsolidation at reinduction I initiation	107 (26)
Postreinduction at reinduction II initiation	90 (22)
Patients with infection outcomes, No. (%)	
Bloodstream infections[a]	26 (13)
Febrile neutropenia	122 (61)
Diarrheal illness[b]	73 (37)
No infection outcome[c]	42 (21)

[a]Etiologic agents of bloodstream infections included coagulase-negative staphylococci (7 episodes), *Escherichia coli* (3), *Enterococcus* species (3, all susceptible to vancomycin), *Staphylococcus aureus* (3), *Streptococcus pneumoniae* (3), *Rothia mucilaginosa* (2), *Neisseria polysaccharea* (2), *Stenotrophomonas maltophilia* (1), *Enterobacter cloacae* (1), *Micrococcus* species (1), *Bacillus cereus* (1), *Pseudomonas aeruginosa* (1), *Streptococcus salivarius* (1), *Capnocytophaga sputigena* (1), and *Burkholderia cepacia* (1).

[b]Etiologies included *Clostridium difficile* (45 episodes), rotavirus (5), norovirus (5), adenovirus (4), and *Cryptosporidium* (2).

[c]No fever, bacteremia, febrile neutropenia, or diarrheal illness.

112 episodes of diarrheal illness (Table 1). Characteristics of patients with samples at each time point are shown in Supplementary Table 1.

Changes in Gut Microbiota Diversity

Microbiome diversity significantly decreased from diagnosis to the end of induction (3.34 [standard deviation {SD}, 0.72] vs 2.82 [SD, 0.88]; $P < .001$; Figure 1 and Supplementary Table 2). However, after less-intensive consolidation and continuation treatment, diversity reverted to the baseline level (3.18 [SD, 0.69] postconsolidation vs 2.82 [SD, 0.88] postinduction; $P < .01$). Gut microbiota diversity again decreased significantly after reinduction I (2.84 [SD, 0.76] postreinduction vs 3.18 [SD, 0.69] postconsolidation; $P < .01$). Although there was no significant difference between the diversity indices at baseline and postconsolidation or between those at postinduction and postreinduction, the index at postreinduction was significantly lower than that at baseline ($P < .001$; Figure 1), suggesting that induction and reinduction chemotherapy reduced the gut microbial diversity. Similar results were shown across all alpha diversity indices (Supplementary Figure 3).

Changes in Gut Microbiota Composition

Compared to baseline, the mean log RA of Bacteroidetes, *Faecalibacterium* species, Ruminococcaceae, Actinobacteria, and Verrucomicrobia significantly decreased in at least one of the postchemotherapy samples (Figure 2 and Supplementary Tables 2 and 3). In contrast, the mean log RA of Clostridiaceae, Streptococcaceae, Lactobacillaceae, Enterococcaceae, and other Firmicutes significantly increased after chemotherapy (Figure 2 and Supplementary Tables 2 and 3). Although mean diversity of the postconsolidation samples did not differ significantly from that of the baseline samples nor did the diversity of the postinduction samples compared to postreinduction samples, the gut microbiome composition changed significantly. The diversity at postconsolidation reverted back to baseline level, but the composition was different.

Effect of Antibiotics and Probiotics on Microbiome

Impact of antibiotics on microbiome changes was examined for patients who had stool specimens collected before and after each phase of chemotherapy. Antibiotic exposure was not significantly associated with changes in diversity for any chemotherapy phase (Supplementary Table 4 and Supplementary Figure 4).

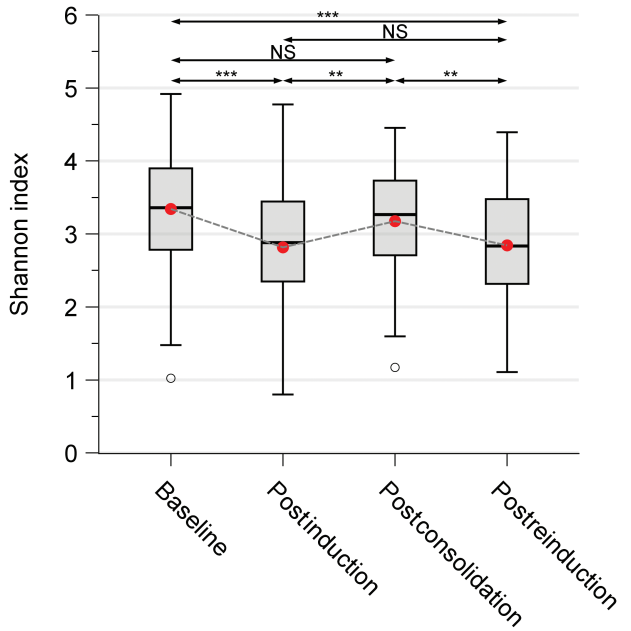

Figure 1. Change in gut microbiota diversity in 199 children during chemotherapy for acute lymphoblastic leukemia. Fecal samples were obtained at 4 time points (baseline, n = 112; postinduction, n = 97; postconsolidation, n = 107; and postreinduction, n = 90). A mixed-effects linear model was used to examine the change in Shannon indices over time. Patients were included as a random effect. $^{**}P < .01$; $^{***}P < .001$. Abbreviation: NS, not significant.

Similarly, no significant association was found between cumulative antibiotic exposure and change in dominance of Bacteroidetes (Supplementary Table 5). Due to the small number (n = 10) of patients who received probiotics at any time, the findings regarding microbiome changes are unlikely to be affected by probiotics (Supplementary Figure 5).

Using the Baseline Microbiome to Predict Any Infection Throughout Therapy

The relationship between baseline microbiome at the time of ALL diagnosis and subsequent development of infection during chemotherapy was characterized. Of the 112 patients with a baseline sample, 65 developed at least one FN episode. There was no significant difference between diversity of baseline samples from patients who did or did not develop FN (median, 3.38 [interquartile range {IQR}, 2.83–4.04] vs 3.34 [IQR, 2.72–3.87]; Supplementary Table 6). However, Proteobacteria were significantly more abundant at baseline in patients who subsequently developed FN (median, 0.03 [IQR, 0.01–0.14] vs 0.01 [IQR, 0–0.06]; $P = 0.027$; Supplementary Table 5). Using an optimal RA cutoff of 0.01%, Proteobacteria RA in baseline microbiome was the only independent predictor of subsequent FN (Supplementary Table 7). After adjusting for sex and the competing risk of death and ALL relapse, the cumulative incidence of first FN episodes in patients with Proteobacteria RA ≥0.01% was significantly higher than in patients with RA <0.01% (hazard ratio [HR], 2.12 [95% confidence interval {CI}, 1.22–3.69]; $P = .008$; Figure 3 and Supplementary Table 7). In summary, the composition, rather than diversity, of baseline gut microbiome was an independent predictor of FN during chemotherapy.

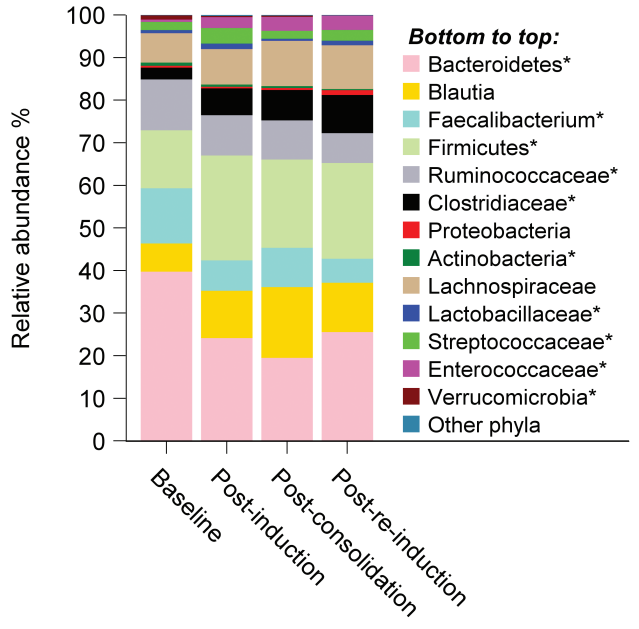

Figure 2. Change in gut microbiota composition during chemotherapy at the same time points as in Figure 1. *Significant change.

Patients presenting at ALL diagnosis with Proteobacteria RA ≥0.01% had a 67% chance of developing FN. The differences found in similar analyses of bloodstream infections and diarrheal illness were not significant (data not shown).

Infections During a Course of Chemotherapy Associated With Gut Microbiome at the Start of That Course

Using all stool samples collected at all 4 time points to generate a heat map and hierarchical clustering, 5 meaningful clusters of composition type were identified: Bacteroidetes-dominant (type 1); dominance of other Firmicutes or Clostridiaceae (type 2); dominance of Enterococcaceae, Streptococcaceae, Lactobacillaceae, or Proteobacteria (type 3); *Blautia* species dominant (type 4); and even distribution of taxa (type 5) (Figure 4). The distribution of the 4 stool-sample time points and the infection outcomes in each cluster are shown in Supplementary Tables 7 and 8. Forty percent of baseline samples were dominated by type 5 cluster and 49% by Bacteroidetes (type 1 cluster). This dominance by Bacteroidetes has previously been shown in the gut microbiome of healthy children and adults [26, 27].

These 5 microbiota composition types at any of the 4 time points, along with diversity, chemotherapy phase, ALL risk level at the time of sample collection, age at ALL diagnosis, sex, and race, were tested to identify risk factors for infection outcomes during chemotherapy phase subsequent to the stool collection time point. Supplementary Table 10 shows that ALL risk level, chemotherapy phase, and time-dependent microbiome composition type were all associated with infection. FN episodes and diarrheal illness were more likely to occur during induction. Similarly, high-risk ALL was significantly associated with an increased risk for diarrheal illness, as compared to standard-risk (HR, 2.16 [95% CI, 1.01–4.59]; $P = .046$) and low-risk ALL (HR, 2.74 [95% CI, 1.26–5.95];

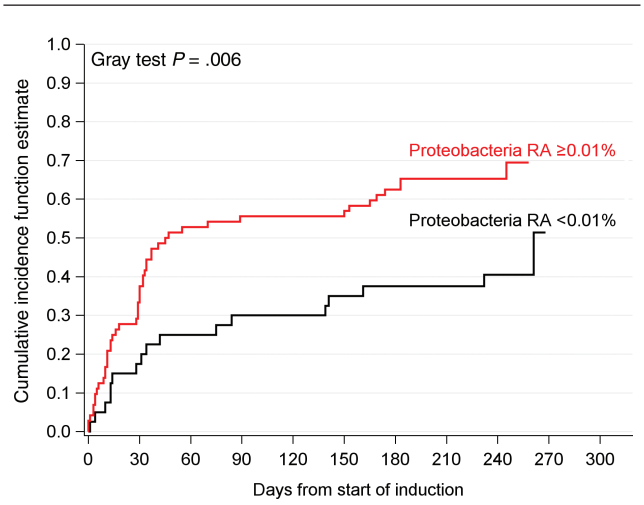

Figure 3. The relative abundance (RA) of Proteobacteria in the baseline gut microbiota at acute lymphoblastic leukemia diagnosis is associated with increased cumulative incidence of first episodes of febrile neutropenia. The estimated hazard ratio was 2.12 (95% confidence interval, 1.22–3.69; $P = .008$) for a Proteobacteria RA of ≥0.01 vs an RA of <0.01%, adjusting for gender.

P = .011). We found no significant association between ALL risk level and FN. Microbiome diversity, age, sex, and race were not associated with FN or diarrheal illness.

Adjusting for chemotherapy phase and ALL risk level, a type 3 gut microbiome composition at any time point was a significant risk factor for FN, diarrheal illness, and any infection outcome (Figure 5). Compared with type 1 and type 5, type 3 composition conferred a higher risk for FN in subsequent chemotherapy phase (HR, 2.40 [95% CI, 1.11–5.20] and 2.88 [95% CI, 1.32–6.28], respectively; Figure 5A). Similarly, type 3 composition was significantly associated with higher risk for diarrheal illness compared with type 1, type 2, type 4, and type 5 (Figure 5B). Diversity was not significantly associated with infection outcomes. We also found no significant association with the time-independent factors, age, sex, and race (data not shown).

Characterization of Type 3 Microbiome Composition

Most (79%) of the fecal samples in the type 3 composition cluster had dominance of Enterococcaceae or Streptococcaceae. Of 14 patients with dominant Enterococcaceae, 7 (50%) developed subsequent infection, as did 4 of 5 (80%) patients with dominant Streptococcaceae (Supplementary Table 11). Adjusting for chemotherapy phase and ALL risk level, Enterococcaceae dominance was significantly associated with increased risk of subsequent FN (HR, 2.97 [95% CI, 1.35–6.53]) and diarrheal illness (HR, 4.23 [95% CI, 1.77–10.1]) compared to nondominance of both Streptococcaceae and Enterococcaceae, while Streptococcaceae dominance carried a greater risk (HR, 7.94 [95% CI, 3.27–19.3]) of subsequent diarrheal illness (Table 2). Thus, a gut microbiota dominated by Enterococcaceae or Streptococcaceae is associated with subsequent infections.

Validation of Enterococcaceae or Streptococcaceae Dominance as Infection Predictor

A bootstrap method confirmed that patients with a gut microbiome dominated by Enterococcaceae or Streptococcaceae had a significantly higher risk of diarrheal illness and any infection outcome (Supplementary Figure 6*A* and 6*B*). Patients with Enterococcaceae or Streptococcaceae dominance also had a higher but nonsignificant risk of FN (Supplementary Figure 6*C*). In summary, the performance of Enterococcaceae or Streptococcaceae dominance as a predictor was validated for diarrheal illness and any infection outcome, and showed a strong trend toward predicting FN.

DISCUSSION

Our study is the first to evaluate changes in the diversity and composition of gut microbiome and its role in predicting infections in a large cohort of children with ALL.

Diversity decreased significantly after intensive induction and reinduction chemotherapy, despite a rebound after recovery from induction. The decreased microbial diversity after immunosuppressive and myelosuppressive treatment is consistent with studies in adult HSCT recipients and acute myeloblastic leukemia or

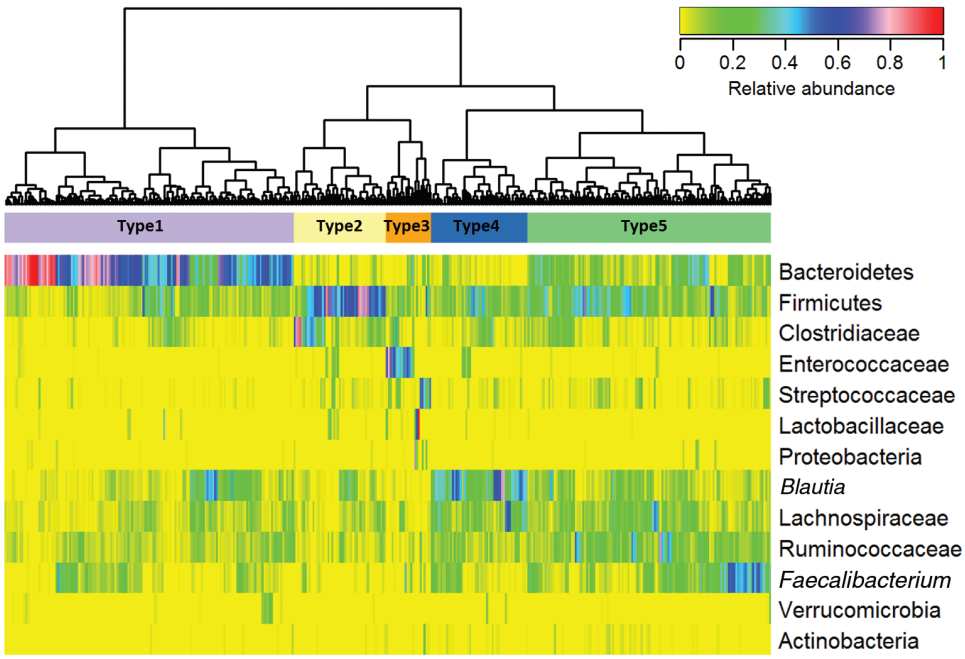

Figure 4. Heat map of taxa relative abundance in microbiota of all 406 fecal samples collected at any of the 4 time points. Five clusters (types 1–5) were identified using unsupervised hierarchical clustering of the Jensen-Shannon divergence matrix and ward linkage. Type 1 is dominated by Bacteroidetes; type 2 by other Firmicutes or Clostridiaceae; type 3 by Enterococcaceae, Streptococcaceae, Lactobacillaceae, or Proteobacteria; and type 4 by *Blautia* species. Type 5 has evenly distributed relative abundances of taxa.

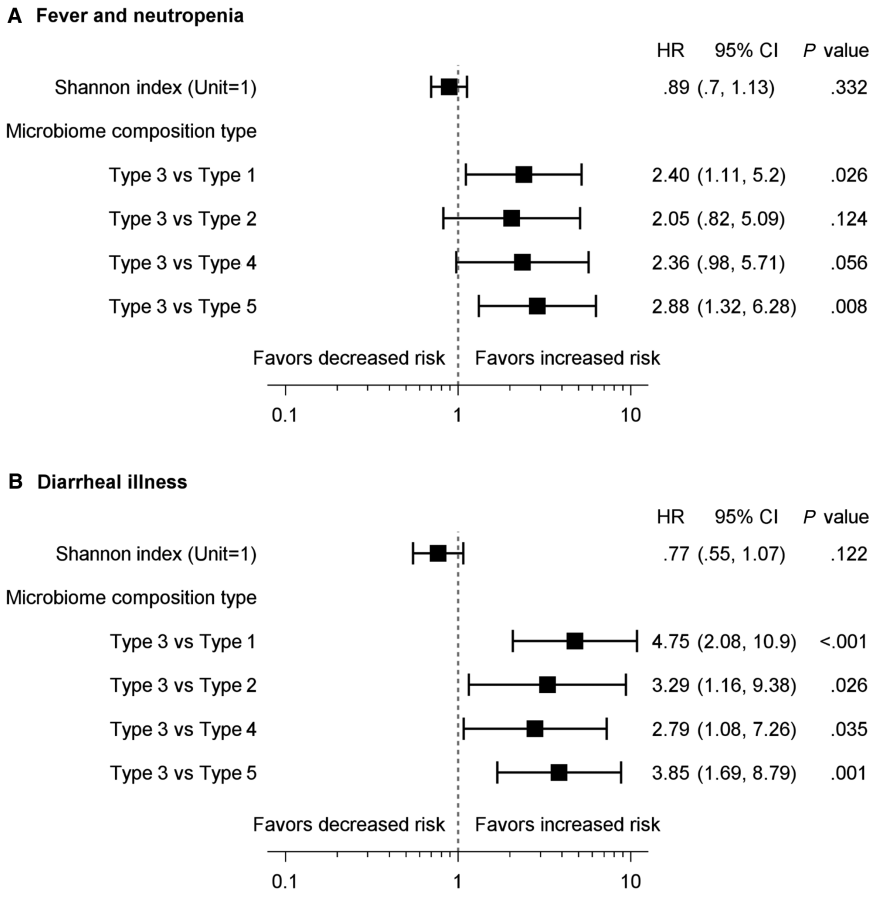

Figure 5. Forest plot of hazard ratios (HRs) with 95% confidence intervals (CIs) for febrile neutropenia (*A*) and diarrheal illness (*B*) associated with gut microbiome composition and diversity at any of the 4 time points. The Anderson-Gill model was used to compare the risk of each outcome among the microbiome Shannon indices and composition types over time. The reported HRs were adjusted for subsequent chemotherapy phase and acute lymphoblastic leukemia risk level, and the 95% CI and *P* values were estimated using the robust sandwich estimator.

non-Hodgkin lymphoma [5, 9, 28, 29]; however, the only other pediatric study to date [11] detected increases in gut microbial diversity during chemotherapy. Although both studies sequenced the V1–V3 regions of the 16S rRNA gene, their divergent findings might be explained by differences in the chemotherapy regimens, antibiotic use, methods of sample preparation and sequencing, data analysis methods, or time points for stool collection.

Although the empirical diversity index recovered to levels similar to those of baseline, this renewed diversity was characterized by a different microbiotal composition. In contrast to the studies in adults, we found that microbiota composition, but not diversity, was independently predictive of infections during chemotherapy. As previously reported [26], the baseline microbiome was dominated by Bacteroidetes; which decreased after

Table 2. Domination of Gut Microbiome at Any Time Point by Streptococcaceae or Enterococcaceae Was Associated With Diarrheal Illness and Febrile Neutropenia in the Subsequent Phase of Chemotherapy

	Associated With FN			Associated With Diarrheal Illness		
Dominance of Microbiome	HR	(95% CI)	*P* Value	HR	(95% CI)	*P* Value
Streptococcaceae-dominant[a] vs Enterococcaceae-dominant[b]	0.63	(.1–4.11)	.628	1.88	(.59–5.96)	.285
Streptococcaceae-dominant vs others[c]	1.87	(.34–10.4)	.475	7.94	(3.27–19.3)	<.001
Enterococcaceae-dominant vs others	2.97	(1.35–6.53)	.007	4.23	(1.77–10.1)	.001

Anderson-Gill model was used to compare the risk of each infection outcome in different groups over time. The reported HRs were adjusted for the phase of chemotherapy and acute lymphoblastic leukemia risk level at the time of stool sample collection as appropriate.

Abbreviations: CI, confidence interval; FN, febrile neutropenia; HR, hazard ratio.

[a]Streptococcaceae-dominant: ≥30% relative abundance (RA) of Streptococcaceae.

[b]Enterococcaceae-dominant: ≥30% RA of Enterococcaceae.

[c]Others: RA <30% for both Streptococcaceae and Enterococcaceae.

chemotherapy. A reduction in Bacteroidetes has been linked to human disease [30]. The metabolic function of some of these bacterial taxa was explored in pathogenesis studies [28, 31]. For example, Ruminococcaceae and *Faecalibacterium* species have been implicated in anti-inflammatory effects and may maintain intestinal epithelial integrity [31]. The loss of the protective effect of these taxa after chemotherapy might compromise gut integrity and increase the risk of bacterial translocation into the bloodstream.

On the other hand, the RA of bacterial taxa that commonly infect immunocompromised patients, such as Streptococcaceae and Enterococcaceae, increased significantly after chemotherapy. This is consistent with a study evaluating 94 adult HSCT recipients whose gut microbiota became dominated by *Enterococcus* species, *Streptococcus* species, and Proteobacteria [9]. Studies have demonstrated that gut microbial communities recover after resolution of perturbing factors [10, 32, 33]. We saw no such recovery of gut microbiota composition at the 3 time points of stool sampling during chemotherapy. We found no association between antibiotic exposure and microbiome changes. As shown in Supplementary Figure 4, all patients received antibiotics during induction chemotherapy, and the majority did during consolidation and/or reinduction. It is not clear whether the change in microbiome is due to antibiotics or the chemotherapy itself. We believe that the confounding between antibiotic administration and the chemotherapy makes it difficult to isolate an effect of antibiotics alone on the microbiota. It would be interesting to examine these patients' microbiomes after completion of chemotherapy and resolution of their immunosuppressed state.

We have shown that the composition, but not diversity, of gut microbiota is a significant predictor of infections. Presentation with a gut characterized by Proteobacteria at ALL diagnosis significantly predicted FN during chemotherapy. The Proteobacteria phylum of gram-negative bacteria includes Enterobacteriaceae, *Pseudomonas* species, and other bacteria commonly causing infections in immunocompromised patients. The importance of Proteobacteria as markers of imbalanced gut microbiota and increased risk for disease has been previously documented in immunocompetent and immunocompromised patients [9, 10, 34].

In addition to the baseline gut microbiome, characterization of the microbiome composition before each phase of chemotherapy independently predicted infections during the upcoming phase. Adjusting for chemotherapy phase and ALL risk level, domination of the gut by Enterococcaceae or Streptococcaceae predicted subsequent FN or diarrheal illness. This is consistent with adult studies showing that an increased abundance of certain taxa (Proteobacteria, *Enterococcus* species, and *Streptococcus* species) was associated with subsequent bloodstream infections and mortality [9, 35].

Our study included the largest pediatric ALL cohort with gut microbiome evaluation, and it represents the most comprehensive analysis of the microbiome's relation to infections. However, it did not adjust for additional factors that might contribute to changes in gut microbiota, including diet, ethnicity, body mass index, residence geographic location, and pet exposure. Also, not all patients provided stool samples at all 4 time points.

In conclusion, we have shown that microbiome composition can be used as an infection risk stratification tool at the time of presentation with ALL diagnosis. If validated, evaluations of the gut microbiome at the onset of chemotherapy could be used to determine biomarkers of the clinical course during chemotherapy. Our findings emphasize the need for enhanced understanding of microbiome in children with cancer during chemotherapy to design targeted microbiome-based diagnostic and biomarker tools. Therefore, once a patient is identified at risk for infections, customized treatment regimens could be designed to mitigate this risk by selectively manipulating the gut microbiota composition using customized probiotics, fecal microbiota transplant, modified diet, or other innovative approaches. However, more studies are needed. Our findings can guide the design of future studies to evaluate the performance of microbiota biomarkers and the proposed treatment regimens that modify gut dysbiosis.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. H. H. designed the study, performed sample and clinical data collection, interpreted the analysis, and wrote the manuscript. R. D. and V. D. contributed to the clinical data abstraction and the sample collection and tracking. S. J. and C.-H. P. contributed to patient recruitment and reviewed the findings and the manuscript. J. W. R., S. S.-C., C. J., and E. A. K. designed and validated the 16S sample-analysis pipeline. T. C. completed the computational analysis. L. T., Y. S., and S. P. designed and performed the statistical analysis. J. W., S. S.-C., R. T. H., E. T., and J. W. R. assisted with results interpretation and with writing and reviewing the manuscript.

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