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Gastrointestinal Microbiome and Mycobiome Changes during Autologous Transplantation for Multiple Myeloma: Results of a Prospective Pilot Study

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Microbiome dysbiosis has been associated with adverse outcomes of hematopoietic cell transplantation (HCT). We hypothesized that exposure to high-dose melphalan and antimicrobials in patients undergoing autologous HCT for plasma cell disorders results in oral and gastrointestinal microbial dysbiosis, which in turn is associated with regimen-related toxicities. We conducted a prospective study describing the longitudinal changes in oral and gastrointestinal bacteriome and mycobiome in this patient population. Our findings show that microbiome composition present at baseline is associated with the incidence and severity of post-transplantation nausea, vomiting, and culture-negative neutropenic fever, as well as with the rate of neutrophil engraftment. We also have evidence of an association between the microbial communities at count nadir and the development of regimen-related gastrointestinal toxicities commonly observed after exposure to high-dose melphalan. Although bacteriome diversity largely recovers within 1 month after transplantation, we observed a continuous decrease in oral and gastrointestinal mycobiome diversity, suggesting that the mycobiome requires a longer time to recover compared with the bacteriome.

INTRODUCTION

Hematopoietic cell transplantation (HCT) is frequently associated with severe toxicities, including graft-versus-host disease (GVHD), gastrointestinal mucositis, and opportunistic infections. Since the 1970s, the microbiome—specifically, intestinal microbiota—and their metabolites have been recognized as key mediators of allogeneic HCT outcomes, including GVHD and overall survival (OS) [1–10]. The microbiome has been shown to influence post-transplantation hematologic reconstitution, including reconstitution of dendritic cells, neutrophils, B cell and T cell subsets, and natural killer cells [11–19]. Despite the rapidly accumulating evidence that the fecal bacteriome plays a key role in allogeneic HCT, less is known about its role in the context of autologous HCT.

A study of patients undergoing autologous HCT for non-Hodgkin lymphoma identified decreases in Firmicutes and Actinobacteria abundance but an increase in Proteobacteria abundance in fecal samples collected after exposure to conditioning compared with baseline fecal samples [20]. Several studies have examined the association of oral microbiome changes and the development of chemotherapy-induced oral mucositis (OM) [21–23]. However, the potential roles and associations of the fecal and oral microbiota and mycobiota with regimen-related toxicities of high-dose therapy and autologous HCT have not yet been comprehensively studied. We hypothesized that exposure to high-dose melphalan and antimicrobials in patients undergoing autologous HCT for plasma cell disorders results in oral and intestinal microbial dysbiosis. We also sought to investigate whether the pattern of microbial disruption correlates with the severity of regimen-related toxicities (specifically OM and gastrointestinal toxicity), engraftment, and infectious outcomes. To test this hypothesis and identify specific microbial signatures, we conducted a

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prospective, nonrandomized pilot study of temporal changes in the oral and lower gastrointestinal bacteriome and mycobiome profiles.

METHODS

Study Design and Participants

We designed a prospective pilot study to examine associations between clinical outcomes and changes in the oral and gut microbiota and mycobiota of adult patients (age ≥18 years) treated with amifostine and high-dose melphalan followed by autologous HCT for a plasma cell disorder. This study was approved by the Institutional Review Board at the University Hospitals Cleveland Medical Center. All subjects provided informed consent.

Oral rinse and fecal samples were collected before and after exposure to high-dose melphalan from 15 eligible patients. The preparative regimen consisted of high-dose melphalan, either 200 mg/m^2 (n = 9) or 140 mg/m^2 (n = 6). Diagnoses were multiple myeloma in 14 patients and POEMS syndrome in 1 patient. Patients received 30 minutes of oral cryotherapy before melphalan infusion. All patients were started on prophylactic fluconazole and acyclovir and received prophylactic ciprofloxacin when the absolute neutrophil count dropped below 500/mm^3. In accordance with institutional protocol, piperacillin-tazobactam was started as first-line broad spectrum antibiotic coverage at the onset of febrile neutropenia.

Clinical Outcomes and Toxicity Assessment

Gastrointestinal toxicities, including OM, nausea, vomiting, and diarrhea, were assessed and scored by 2 independent clinicians on a daily basis according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 (Supplementary Table 1). Changes in patient-reported OM quality-of-life (OMQoL) scores were recorded [24], and scores were correlated with the objective CTCAE grading system. Patients consented to complete a 31-question OMQoL designed to assess symptom severity and distress caused by OM. Measures included impact on diet and taste changes, social function, and swallowing. The questionnaire was administered on admission at the day of transplantation and then every third day until discharge. A higher score indicates worsening QoL. Data analysis was performed using R version 3.3.0 (R Institute for Statistical Computing, Vienna, Austria). A linear mixed model was used for longitudinal data analysis to compare the percentage change in the OMQoL score/severity to baseline on admission. The OMQoL score was correlated with CTCAE OM grade severity using a simple linear regression model.

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count ≥500 cells/mm^3 (0.5 × 10^9/L).

Sample Collection and Specimen Processing

Oral rinse samples were collected at least 1 hour after a meal, at approximately the same time (9 to 11 AM) to avoid contamination of samples with extraneous components and to standardize the impact of variation in salivary flow rates. The samples were obtained by having the participants swirl 10 mL of sterile normal saline in their mouth for 1 minute and then expectorate the fluid into a 50-mL centrifuge tube. Samples were collected on days -2, +7, and +30 (with day 0 being the day of stem cell infusion/transplantation). Oral rinse samples were stored at -80°C until the DNA extraction procedure.

Fecal samples were collected in sterile screw top containers and sent to the lab within 2 hours of collection. Samples were collected on days -2, +7, and +30. If samples could not be sent to the laboratory within 2 hours (i.e., after 5 PM and during weekends), they were refrigerated at 4°C until the next business day. Then 300 mg of fecal aliquot was placed in sterile Eppendorf tubes and stored at -80°C until further analysis. Similarly, if not feasible due to logistics, samples were collected within 1 to 5 days of these preplanned time points.

DNA Extraction

DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Oral samples were centrifuged, and the resulting pellets were transferred to tubes with 1 mL of InhibitEX lysis buffer (Qiagen). Similarly, all fecal swabs were transferred, incubated for 1 hour at 75°C, and shaken using FastPrep 96 twice for 300 seconds each at a speed of 1800 rpm. Equal amounts of 100% ethanol and lysate were mixed in a collecting tube and passed through HiBind DNA Mini Columns (Omega Bio-tek, Norcross, GA), and the resulting DNA pellet was eluted using 50 μL of molecular grade water. The quality and purity of the isolated genomic DNA were confirmed by gel electrophoresis and quantitated with a Qubit 2.0 fluorometer applying the Qubit dsDNA HS Assay (Thermo Fisher Scientific, Waltham, MA). DNA samples were stored at -20 °C.

PCR Amplification

Amplifications of the 16S and 5.8S rRNA genes were performed using 16S-804 (5′-(TCC TAC GGG AGG CAG CAGT-3′) and 16S-515 (5′-GGA CTA CCA GGG TAT CTA ATC CTG-3′) and ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) primers, respectively. The PCR mixture was composed of Q5 High-Fidelity Master Mix (New England Biolabs) at a 1× concentration, along with a double volume of molecular grade water and 0.05 μL/mM of each primer. Undiluted DNA (1.5 μL) was added to each 50 μL reaction. Thermocycling conditions consisted of an initial denaturation step (3 minutes at 98°C), followed by 30 cycles of denaturation (10 seconds at 98°C), annealing (10 seconds at 55°C for the 16S primers and 20 seconds at 58°C for the ITS primers), extension (10 seconds at 72°C), and a final extension step of 3 minutes at 72°C. Then 10 μL of each PCR product were separated using gel electrophoresis on 1.5% agarose gel (containing 7 μg/mL ethidium bromide).

Library Preparation and Sequencing

The amplicon library was cleaned and barcoded, followed by emulsion PCR using the Ion Torrent next-generation sequencing data analysis workflow (Thermo Fisher Scientific) according to the manufacturer's instructions. Equal volumes of bacterial 16S rRNA and fungal ITS amplicons were pooled, cleaned with AMPure XP beads (Beckman Coulter, Brea, CA) to remove unused primers, and then exposed to end-repair enzyme for 20 minutes at room temperature. After an additional AMPure cleanup, ligation was performed at 25°C for 30 minutes using Ion Torrent P1 and a unique barcoded "A" adaptor per pooled sample. After AMPure removal of residual adaptors, samples were concentrated to 1/4 volume for 1 hour using vacuum (Labconco, Kansas City, MO) under heat. All separate barcoded samples were then pooled in equal amounts (10 μL) and size-selected for the anticipated 16S and ITS range (200 to 800 bp) using Pippin Prep (Sage Bioscience, Beverly, MA). The library was amplified for 7 cycles and then quantitated on a StepOne qPCR instrument (Thermo Fisher Scientific) ahead of proper dilution to 300 pM going into the IonSphere templating reaction on the Ion Chef (Thermo Fisher Scientific). Library sequencing was completed on an Ion Torrent S5 sequencer (Thermo Fisher Scientific), and barcode-sorted samples were analyzed in our custom pipeline based on Greengenes V13_8 and Unite database V7.2, designed for the taxonomic classification of 16SrRNA and ITS sequences, respectively. Sequencing reads were clustered into operational taxonomic units (3% distance), described by community metrics and taxonomically classified within the Qiime bioinformatics pipeline.

Statistical Analysis

A custom pipeline based on Greengenes V13_8 and Unite database V7.2 was designed for the taxonomic classification of 16SrRNA and ITS sequences, respectively. Downstream data analysis was performed using Qiime [25]. Statistical analysis was performed using R version 3.5.0.

Change across time in phyla and genus abundance at the community level were assessed using the nonparametric multivariate distance-based analysis of variance using the Bray-Curtis (BC)distance for the dissimilarity metric along with its standardized binary form.

Diversity was analyzed in an unbiased manner using the Shannon diversity index, a measure of abundance taking microbial distribution into account. Richness was also assessed, reflecting the microbial counts of the bacterial and fungal communities in each sample.

Nonparametric multivariate distance-based associations between bacterial or fungal communities and outcomes were performed using the adonis function as implemented in the R vegan package version 2.5.2 using Bray-Curtis dissimilarity distance metric and its standardized version (SBC) based on presence/absence instead of on abundance. Of note, adonis testing shows correlation without directionality. Nonparametric Spearman's correlation and Wilcoxon's rank-sum test were used for associations with continuous outcomes and binary outcomes, respectively.

Longitudinal analysis was performed using all pairwise multiple comparison of mean ranks as implemented in the PMCMR plus R package version 1.2.0, using the Kruskal-Wallis test followed by Bonferroni-Dunn post hoc adjustment. $P < .05$ was considered statistically significant for all tests after correcting for multiple comparisons. Correction for multiple testing was performed using Benjamini-Hochberg adjustment for multiple testing.

RESULTS

Patient Demographics and Clinical Outcomes

Oral rinse and fecal samples were collected from all 15 enrolled patients (Table 1). There were no treatment-related deaths, and all patients were alive at 1 year after transplantation. The mean time to neutrophil engraftment was 11 days (range, 9 to 18 days). Culture-negative febrile episodes occurred in 11 patients; 1 patient developed bacteremia, 1 had a urinary tract infection, and 1 had *Clostridium difficile* infection. No patient required parenteral nutrition or patient-controlled analgesia for control of OM or gastrointestinal toxicities. Grade 2-4 OM was experienced by 4 patients (27%),

Table 1
Patient Demographic and Clinical Characteristics (N = 15)

Variable	Value
Age, yr	
Median	62
Range	41-77
Sex, n	
Female	4
Male	11
Ethnicity, n	
African American	5
Caucasian	10
Disease, n	
Multiple myeloma	14
POEMS	1
Subtype, n	
IgG λ	5
IgG κ	5
IgA λ	1
IgA κ	2
λ light chain	1
Stage, n	
R-ISS I	3
R-ISS II	3
R-ISS IIII	3
Unknown	5
Melphalan, n	
140 mg/m^2	6
200 mg/m^2	8
Time to neutrophil engraftment, d	
Mean	11
Range	9-18
Infection, n	
Febrile neutropenia	11
Bacteremia	1
Pulmonary complications	0
Urinary tract Infections	1
Clostridium difficile	1
None	4

R-ISS indicates Revised International Staging System.

and grade 2-4 nausea, vomiting, and diarrhea were reported in 9 (60%), 4 (27%), and 8 (52%) patients, respectively. The majority of toxicities were grade 2 (Supplementary Table 1).

Fourteen of the 15 patients completed the OMQoL questionnaire; 1 patient withdrew consent. OMQoL scores increased progressively from baseline on day -2 to day +3, day +6, and reaching a peak score and severity on day +9 ($P < .001$). These changes coincided with marrow aplasia, which subsequently improved on day +12 ($P < .001$). We observed a positive correlation between patient-reported decline in OMQoL and objective measurements of OM severity, graded according to CTCAE on day +6 ($P < .001$) and day +9 ($P < .001$).

Longitudinal Changes in Bacterial and Fungal Composition and Relative Abundance

A total of 42 oral rinse and 42 fecal samples were collected. Three patients withdrew consent before day +30, resulting in the loss of 3 oral samples and 3 stool samples. The total number of bacteriome and mycobiome reads was 3.3 million; after passing through quality filters, sequences were used for operational taxonomic unit assignments. Taxa at the phylum and genus levels with the sum of abundance across all fecal and oral samples <10 were filtered out and excluded from the statistical analysis. Relative abundances of bacterial and fungal phyla and genera are presented in Supplementary Tables 2 to 5. The number of reads for each sample are detailed in Supplementary Tables 2 to 4.

Bacteriome

Figures 1 and 2 illustrate the changes over the 3 time points for the 4 most abundant oral and fecal bacterial species at the phylum and genus levels, respectively.

At the bacterial phylum level, the oral bacterial community composition changed significantly on day +30 compared with baseline (BC, $P = .046$) and day +7 (BC, $P = .025$). No other statistically significant differences were noted.

We next explored individual bacterial phylum and genus changes across time points using the Wilcoxon pairwise test.

Comparing oral samples collected on days +7 to +30, we found that Proteobacteria decreased and Firmicutes increased with borderline significance after post hoc adjustments ($P = .08$ and .09, respectively). The oral phyla Firmicutes increased from baseline to day +30 (Figure 1; $P = .04$). Comparing baseline oral samples with day +7 samples showed an increase in *Streptococcus* (Figure 2), with borderline significance after post hoc adjustment ($P = .06$). The relative abundance of fecal *Bacteroides* (Figure 2; $P = .03$) increased on day +30 after a drop on day +7, but the change did not maintain significance after multivariate adjustment.

Mycobiome

When examining changes in mycobiome composition, the nonparametric multivariate distance-based test showed a significant difference in the fecal fungal genus between baseline and day +30 (SBC, $P = .025$). No statistically significant differences were noted at the phylum level across any 2 time points.

Figures 3 and 4 illustrate the changes over the 3 time points for the 4 most abundant oral and fecal fungal species at the phylum and genus levels, respectively.

Analysis of the individual fungal phyla and genera using Wilcoxon pairwise analysis across time points with post hoc adjustment revealed that the fecal genus *Fusarium* ($P = .03$) decreased between samples collected on day -2 and those collected on day +7 (Supplementary Figure 1). The oral genus *Glomerella* ($P = .04$) decreased from baseline to day +30.

Bacterial and Fungal Richness and Diversity Analysis

In oral samples, bacteriome genus diversity was slightly increased on day +7 and subsequently returned to pretransplantation levels in the samples collected on day +30, whereas richness at the sample level increased on day +30 without reaching statistical significance. In fecal samples, the bacteriome diversity decreased on day +7 after exposure to the conditioning regimen, with an increase noted on day +30 without reaching baseline levels or statistical significance. This change in diversity is also reflected in a parallel pattern of change in fecal sample richness, without reaching statistical significance (Figure 5).

The diversity of the oral mycobiome decreased on day +7 during marrow aplasia compared with pretransplantation levels on day -2, with a further decrease noted on day +30. A similar trend was also noted in fungal genus richness in oral samples, without reaching statistical significance. Further analysis of the mycobiome in fecal samples showed a statistically significant decrease in diversity and richness from baseline to day +7 (day -2 versus day +7, $P = .05$), whereas sample richness

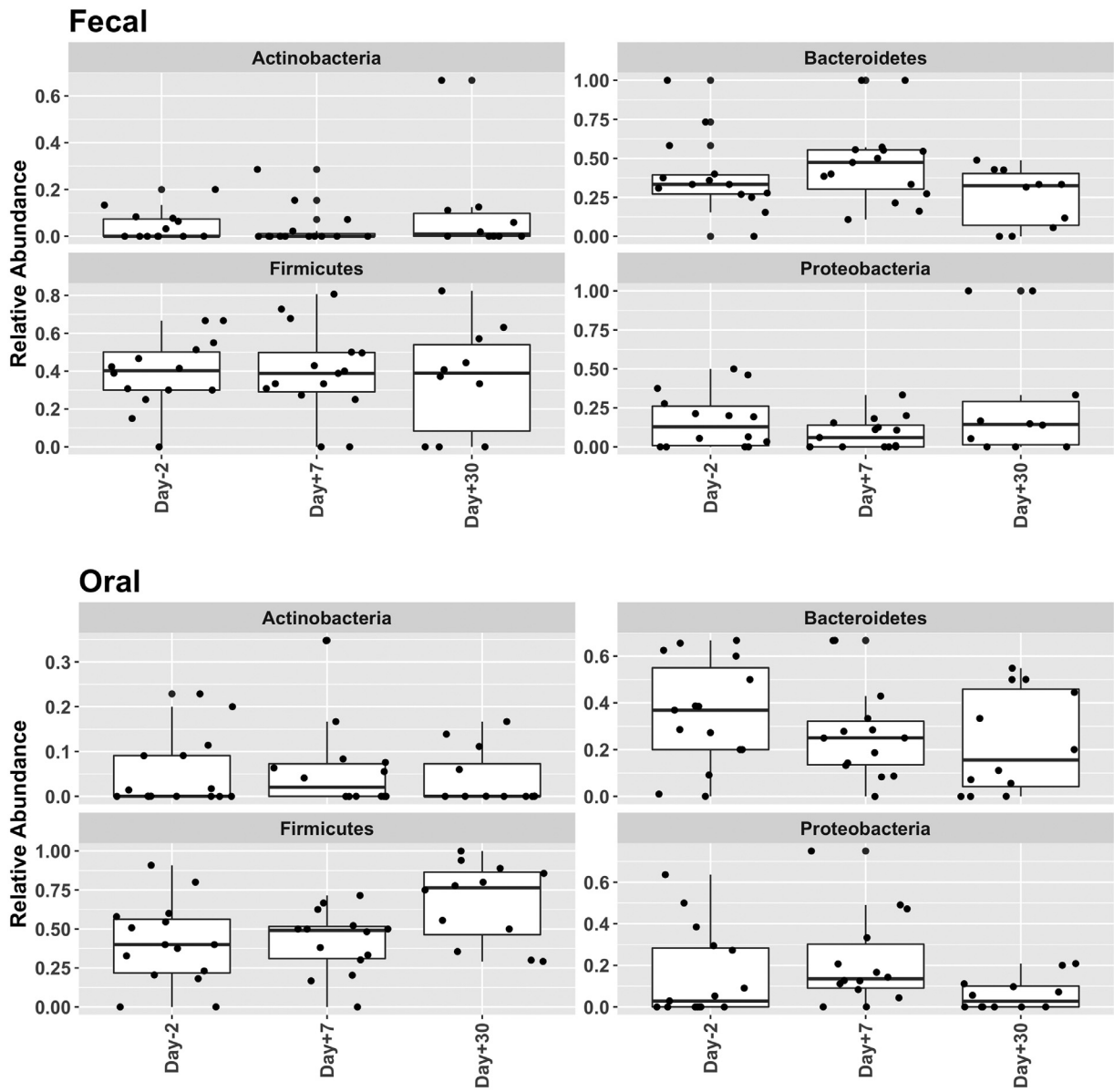

Figure 1. Boxplots of changes in relative abundance of the fecal and oral bacteriomes at the phylum level. The 4 most abundant are shown here.

trended toward significance ($P = .06$). Further decreases in both diversity and richness were noted on day +30.

Correlation of Microbial Diversity and Richness with Clinical Outcomes

We next examined whether a relationship existed between bacterial and fungal diversity and the severity of regimen-related toxicities (specifically OM, nausea, vomiting, and diarrhea), neutrophil engraftment, and the incidence of culture-negative neutropenic fever. We also assessed the effect of exposure to anaerobic-targeting antibiotics on the microbial community.

Bacteriome

In fecal samples, bacterial diversity noted on day +7 during count nadir was associated with the severity of diarrhea experienced by our patients after myeloablation, with lower diversity correlated with more severe diarrhea according to CTCAE scoring ($P = .03$). No significant associations were noted in the oral bacteriome analysis. Anaerobic-targeting antibiotic exposure on or before day +7 affected both genus diversity and richness of the fecal sample collected on day +7 ($P = .015$ and .014, respectively). High bacterial genus richness at baseline ($P = .03$) as well as the diversity and richness noted on day +7 ($P = .01$) during hematologic count nadir was associated with the development of fever on or after day +7 in our cohort.

Mycobiome

When investigating the oral mycobiome, exposure to anaerobic-depleting antibiotics correlated positively with the richness of fungal genus in samples collected around day +30 ($P = .04$). No significant associations were noted in the fecal mycobiome analysis; however, there was a trend towards significance between the diversity of fecal samples noted at baseline and subsequent development of nausea after transplantation, such that higher diversity was associated with lower incidence and severity of nausea ($P = .06$).

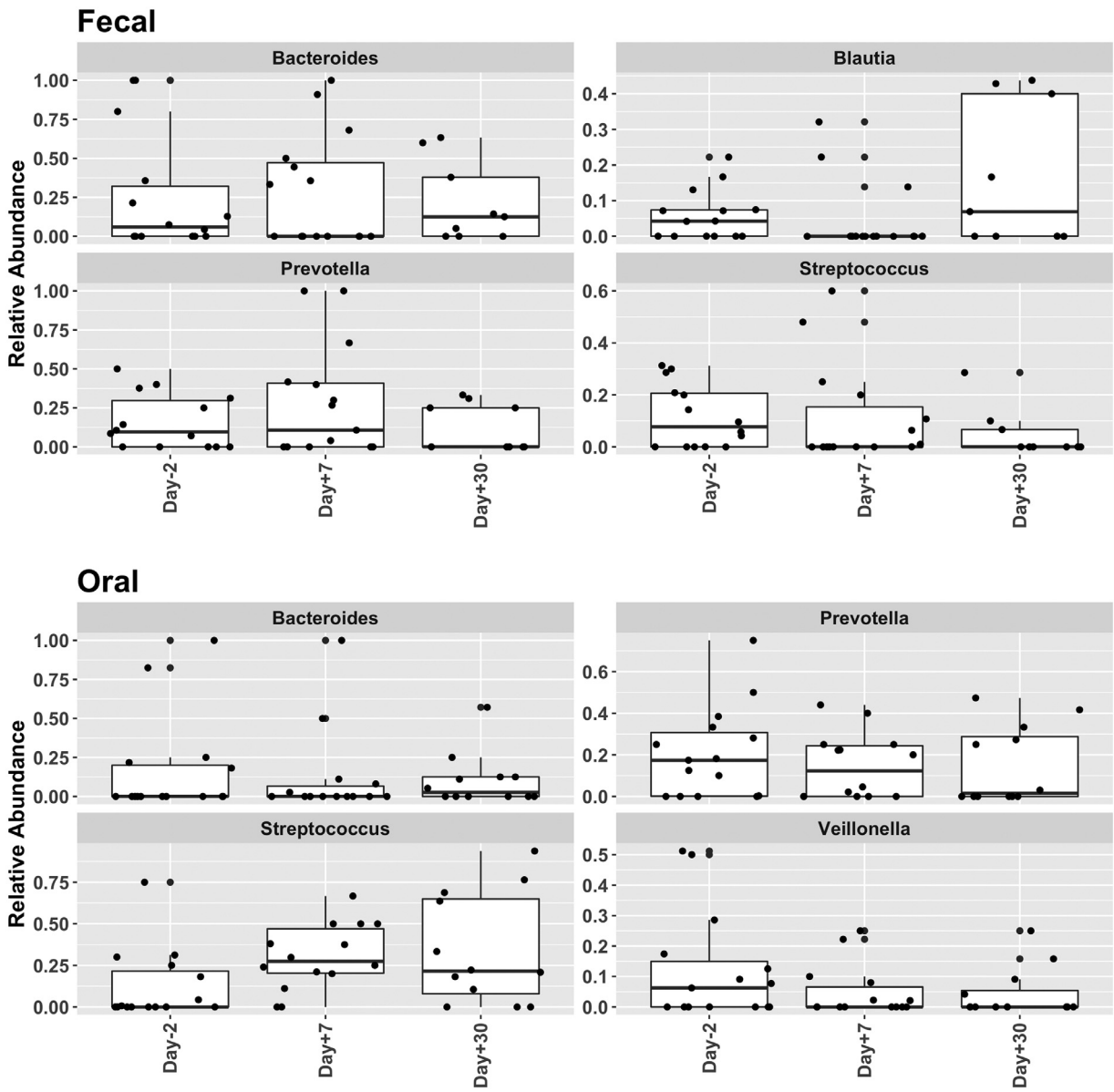

Figure 2. Boxplots of changes in relative abundance of the fecal and oral bacteriomes at the genus level. The 4 most abundant are shown here.

Correlation of Microbial Abundance and Individual Composition with Clinical Outcome and Regimen-Related Toxicity

Associations between microbial abundance and clinical outcomes were tested at the community level and individual taxa level using nonparametric multivariate (distance-based analysis of variance) and univariate (Spearman correlation) analysis at each time points.

Bacteriome

The oral bacteriome community at the phylum level present at baseline was significantly associated with the development and severity of nausea (SBC, $P = .03$), and the bacterial genus present at baseline was significantly associated with the rate of neutrophil engraftment (BC, $P = .05$).

Analysis of the fecal bacteriome showed that the phyla present on day +7 correlated with the incidence of nausea (SBC, $P = .003$; BC, $P = .015$) and diarrhea (BC, $P = .015$ and .009, respectively), as well as with the development of culture-negative neutropenic fever on or after day +7 (BC, $P = .003$). The exposure to anaerobic-targeting antimicrobials on or before day +7 affected the bacterial phyla recovered in fecal samples on day +7 (SBC, $P = .024$).

We further explored a possible correlation between individual bacterial phyla and/or genus on the aforementioned clinical outcomes. In fecal samples, the phylum Bacteroidetes present on day +7 was associated with the development and severity of diarrhea, such that patients with higher abundance of Bacteroidetes experienced lower gastrointestinal toxicity ($P = .03$). In addition, the genera *Blautia* and *Ruminococcus*, belonging to the Firmicutes phylum and Clostridium class, when detected on day +7 were associated with a increased development and severity of vomiting after exposure to high-dose melphalan ($P = .05$ for both, respectively). Presence of the phylum Tenericutes in the oral bacteriome at baseline was associated with a trend toward a protective effect on the incidence and severity of nausea ($P = .01$; $P_{adj} = .06$). Further analysis of the fecal bacteriome present at

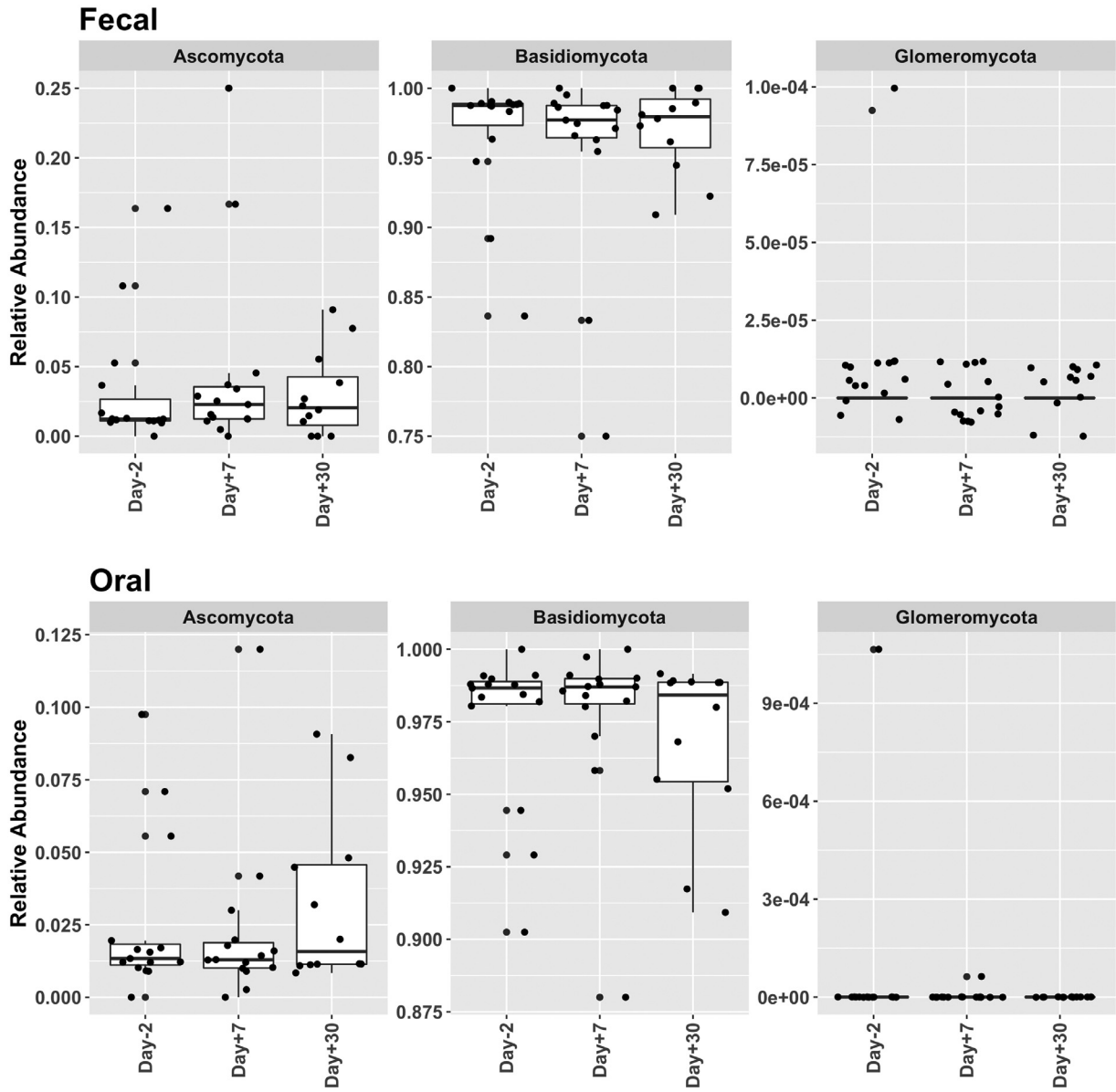

Figure 3. Boxplots of changes in relative abundance of the fecal and oral mycobiomes at the phylum level. The 3 most abundant are shown here.

count nadir pointed to a possible protective effect of Bacteroidetes as opposed to Proteobacteria, which was associated with a trend toward greater severity of nausea in the post-transplantation setting, but without reaching significance after multiple test correction (P = .02; P_{adj} = .06 and P = .01; P_{adj} = .06, respectively).

Mycobiome

The oral fungal genus community present at baseline was associated with the development and incidence of vomiting after transplantation (BC P = .021), as well as the development of culture-negative neutropenic fever before day +7 (BC P= .028). In addition, the genus community present on day +7 was associated with OM incidence and severity (SBC P = .046). Exposure to anaerobic-depleting antimicrobials before day +30 affected the oral fungal genus community identified in day +30 samples (BC P = .007). In fecal samples, the genus community present at baseline (SBC P = .014; BC P = .037) and day +7 (BC P = .03) correlated with the subsequent development of culture-negative neutropenic fevers on or after day +7.

We next investigated whether any individual fungal phyla and/or genus were correlated with clinical outcomes. In oral samples, the presence of the genus *Glomerella* on day +7 was negatively associated with the time to neutrophil engraftment (P = .03).

DISCUSSION

Our study shows that the microbiome composition present at baseline is associated with the incidence and severity of post-transplantation nausea, vomiting, and culture-negative and neutropenic fever and with the rate of neutrophil engraftment. We also have evidence of an association between the microbial communities at count nadir and the development of regimen-related gastrointestinal toxicities commonly observed after exposure to high-dose melphalan. The concomitant analysis of the microbiome and mycobiome in the context

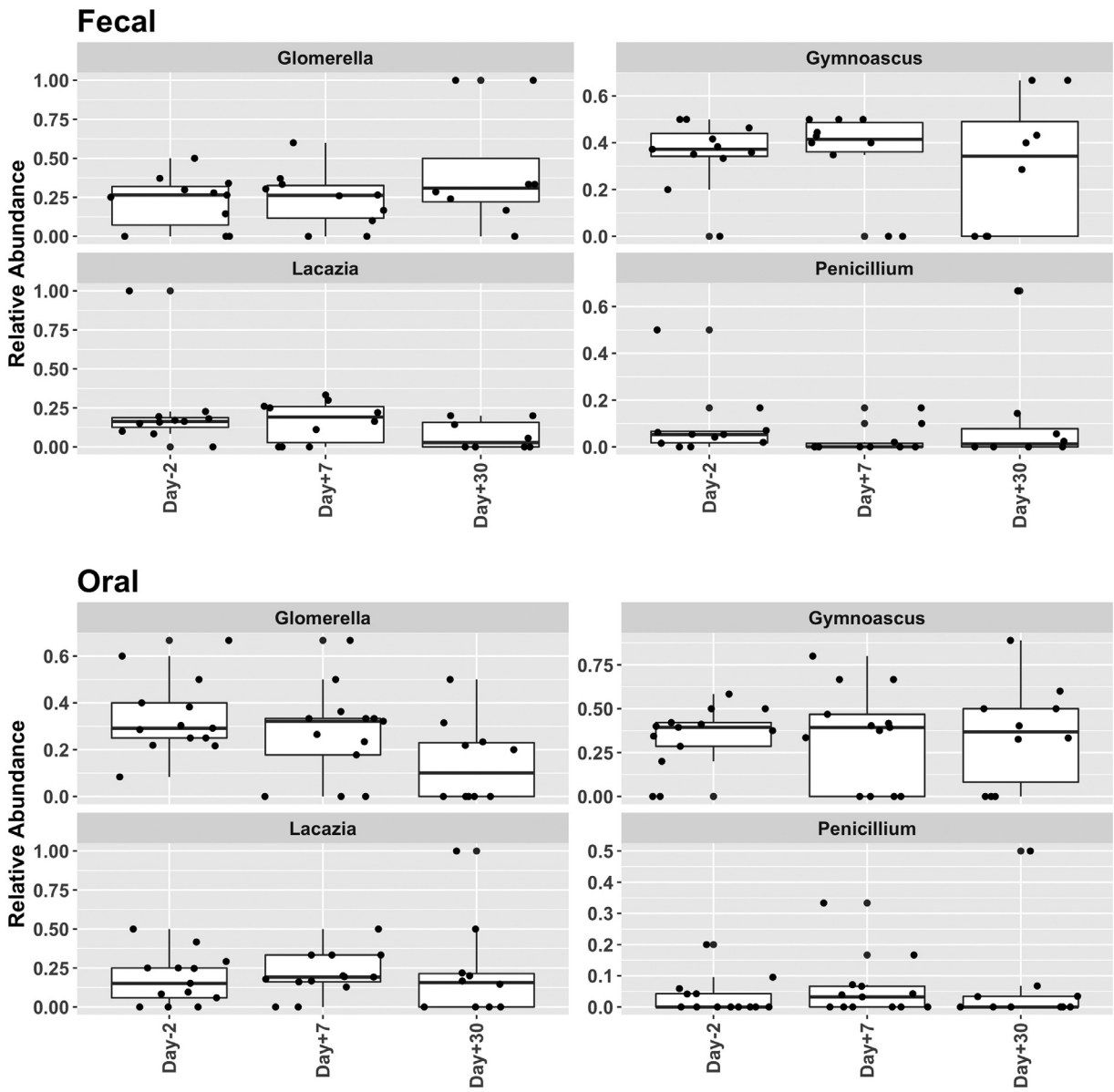

Figure 4. Boxplots of changes in relative abundance of the fecal and oral mycobiomes at the genus level. The 4 most abundant species are shown here.

of autologous transplantation is novel and provides a reference for future interventions.

The oral bacterial intrasample diversity and richness did not change significantly with time. Interestingly, the community composition at the phylum level was significantly different on day +30 compared with that present at baseline and at the hematologic count nadir. This change may reflect a late effect in the oral bacteriome after exposure to chemotherapy and antimicrobials rather than a short-term one. *Streptococcus* increased on day +7 compared with baseline before decreasing again by day +30. It is possible this reflects the use of prophylactic ciprofloxacin initiated on neutropenia in our institution. Interestingly, *Streptococcus mitis* has been identified as a predominant pathogen in patients with breast cancer after exposure to chemotherapy [26]. The oral bacteriome community at the phylum level present at baseline was associated with development and severity of nausea. Despite the small sample size, the bacterial genus composition present at baseline was associated with the time to neutrophil engraftment.

The diversity and richness of the oral mycobiome were decreased on day +7 during marrow aplasia compared with pretransplantation levels, with a further decrease noted on day +30. Exposure to anaerobic-depleting antimicrobials affected the oral fungal genus community identified at 30 days post-transplantation. The oral fungal genus community present at baseline was associated with the incidence of vomiting post-transplantation, as well as with the development of early culture-negative neutropenic fever. In addition, the genus community present on day +7 was associated with the incidence and severity of mucositis, with the peak OM score on day +9. Interestingly, the presence of the genus *Glomerella* on day +7 was negatively associated with time to neutrophil engraftment. *Glomerella* is a known plant pathogen with an as-yet unidentified role in human disease states.

The lower gastrointestinal bacteriome's diversity and richness decreased on day +7, as might be expected after conditioning regimen and antimicrobial exposure. An increase was noted on day +30, but without recovery to baseline levels.

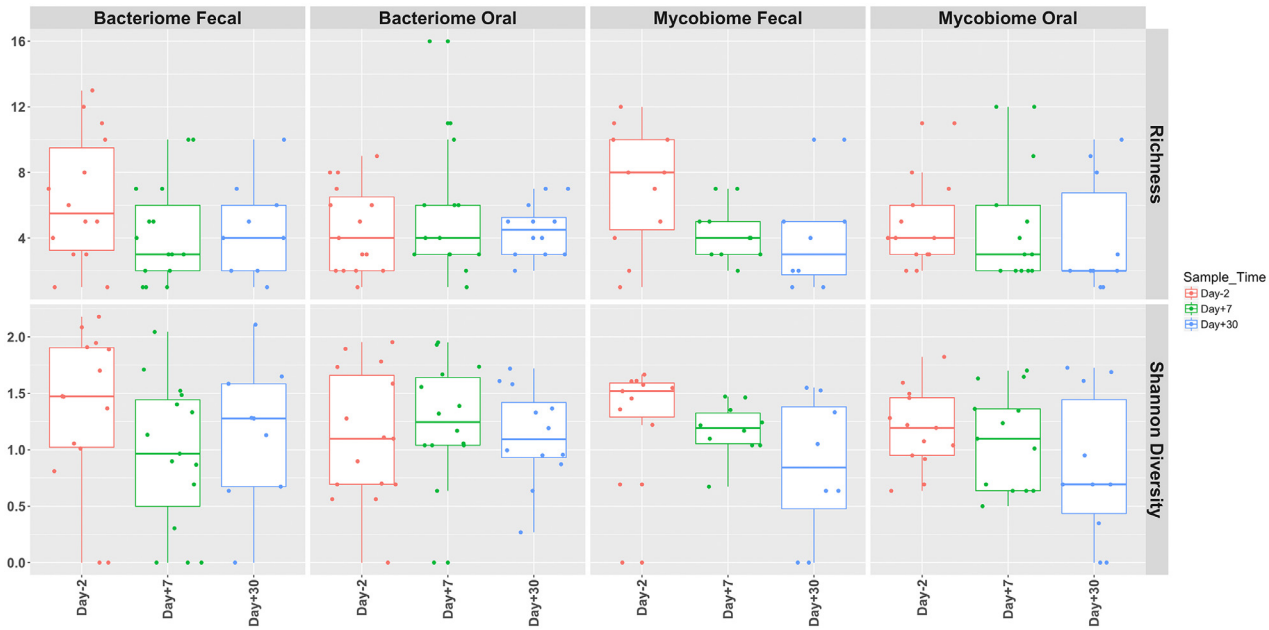

Figure 5. Boxplots of genus diversity and richness of the oral and fecal bacteriomes and mycobiomes.

Anaerobic-targeting antibiotic exposure on or before day +7 affected both the genus diversity and richness of fecal bacteriome noted on day +7, as well as the bacterial phyla recovered in fecal samples on day +7. Interestingly, the bacteriome at the hematologic count nadir was associated with severity of diarrhea. Lower diversity correlated with more severe toxicity. In addition, the collective fecal bacterial phyla community present at day +7 was correlated with the incidence of nausea and diarrhea, as well as with the development of culture-negative neutropenic fever on or after day +7. Specifically, an increased abundance of Bacteroides was associated with a higher rate of lower gastrointestinal toxicity. Bacteroidetes is a gram-negative obligate anaerobic phylum composing up a substantial portion of the human gastrointestinal flora, and a transient increase on day +7 coincided with the peak incidence and severity of gastrointestinal toxicity experienced after high-dose chemotherapy. In addition, *Blautia* and *Ruminococcus*, belonging to the Firmicutes phylum and anaerobic Clostridium class, were associated with a higher frequency and severity of vomiting. However, these genera were present only in low abundance, making the clinical relevance of such a finding questionable and warranting further investigation.

There was a gradual decrease in diversity from baseline to day +7 in the fecal mycobiome, with a further decrease noted on day +30. This similar trend of a continuous decrease in mycobiome diversity was noted in the oral flora as well, suggesting that the mycobiome needs longer to recover compared with the bacteriome. At the phylum and genus levels, the fecal mycobiome genus community differed between baseline and day +30. Interestingly, the genus community present at baseline and on day +7 correlated with the subsequent development of culture-negative neutropenic fever.

Of note, in our institution, amifostine is routinely used as a cytoprotectant before high-dose melphalan and autologous HCT, with an overall reduced rate of mucositis and diarrhea compared with historical controls treated in other institutions [27]. The effect of this organic thiophosphate on the oral and gastrointestinal microbiome is unknown. OM is a frequently encountered toxicity in transplantation, with significant morbidity and effects on both quality of life and health care costs.

Our present findings suggest that changes in the bacteriome and mycobiome are associated with early toxicity after autologous HCT for plasma cell dyscrasias, within the constraints of this pilot study's small sample size. Further studies incorporating metabolomics and proteomics will help elucidate the interactions between the host, the microbiome and mycobiome, and regimen-related toxicities as well as other short-term and long-term transplantation outcomes in the context of autologous HCT.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.04.007.

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