The oral microbiome in pediatric hematopoietic cell transplant recipients

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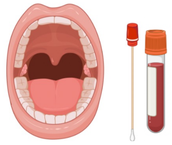
PURPOSE

Oral mucositis (OM) is a toxic side effect during hematopoietic cell transplant (HCT) and a risk factor for bloodstream infections (BSI) as the compromised mucosa becomes susceptible sites for bacterial translocation. Higher incidences of OM and BSI are observed with poor oral health and oral microbial dysbiosis is suggested to be associated with OM, but the specific role of the oral microbiome in OM and BSI pathogenesis is not fully understood. We aimed to characterize the oral microbiome in HCT recipients and investigate its role in the development and progression of OM and BSI.

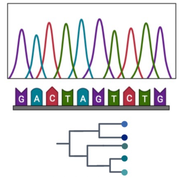
METHODS

Subject recruitment



Clinical exam and sample collection

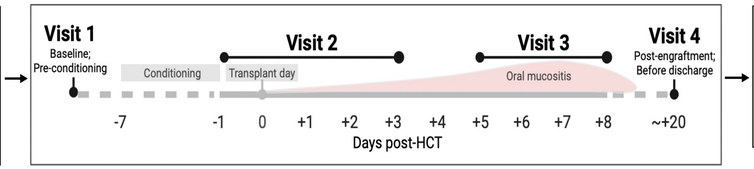
At **each** visit:
- Clinical variables (oral indices, OM score, BSI occurrence) and metadata
- Oral samples (plaque, buccal mucosa, tongue)
- Additional blood and oral samples when (+) BSI



Microbiome sequencing

Inclusion criteria
- Patients undergoing HCT at NCH and receiving myeloablative conditioning regimen

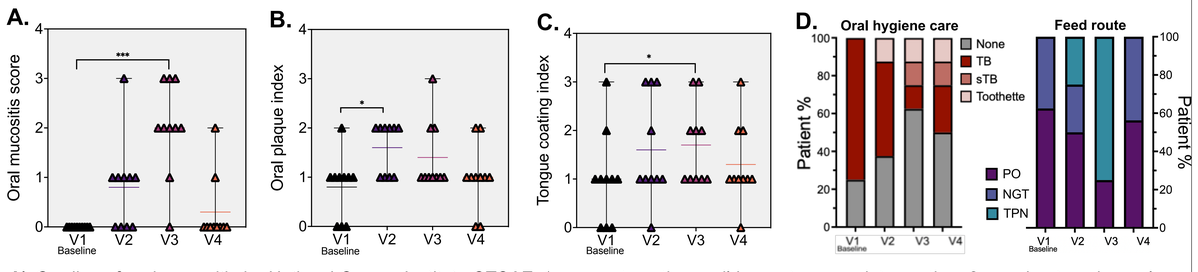
Exclusion criteria
- Patients receiving other conditioning regimens



- 16S rRNA (bacterial) and ITS2 (fungal) sequencing
- If indicated: 16-23S ISR sequencing on selected samples
- qPCR quantification of total bacteria and fungi

RESULTS

Progression of oral mucositis and decrease in oral indices, oral care and PO intake observed post-HCT



A) *Grading of oral mucositis by National Cancer Institute CTCAE:* 1= asymptomatic or mild symptoms, no intervention, 2= moderate pain or ulcer that doesn't interfere with oral intake; modified diet indicated, 3= severe pain, interfering with oral intake. **B)** *Oral plaque index*: amount of tooth surface covered by debris/stain: 0= none, 1= ≤1/3, 2= >1/3 to ≤2/3, 3= >2/3. **C)** *Miyazaki tongue coating index*: extent of dorsal tongue surface coating: 0= none, 1= <1/3, 2= <2/3, 3= >2/3. **D)** Oral hygiene care and feed route: TB: toothbrush + F toothpaste, sTB: toothbrush+ saline, Toothette with saline. PO: by mouth, NGT: Nasogastric tube. TPN: Total parenteral nutrition.
Asterisk: statistically significant differences when compared to V1, baseline. * $p < .05$, *** $p \leq .001$

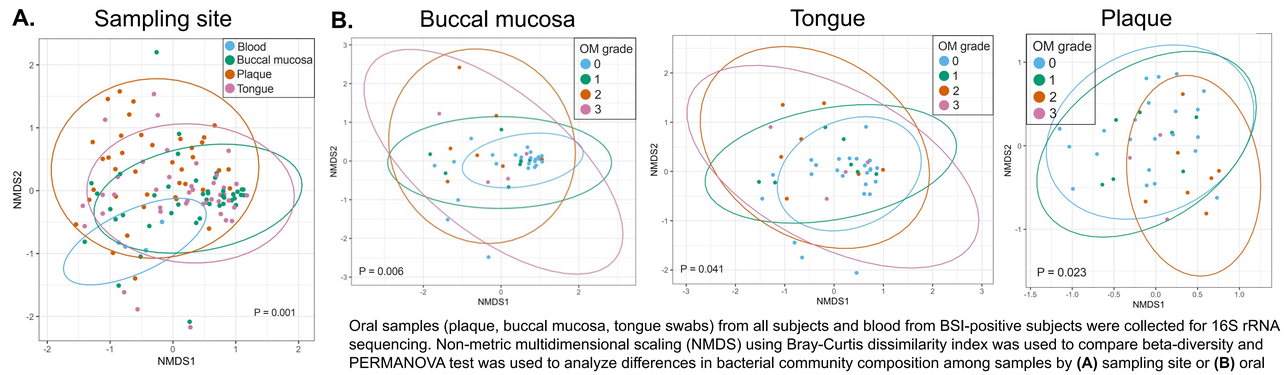
FUTURE DIRECTIONS

1) Expand our sample size to allow the global microbial changes to be elucidated at the *species* level for both bacteria and fungi, **2A)** Comprehensively define the bloodstream microbial profile in children receiving HCT by longitudinally collecting blood samples from all subjects and comparing their bloodstream microbial profile when +/- BSI, **2B)** Determine if the oral cavity (plaque, tongue, mucosa) is the likely source of BSI

This research was supported by:
- ***Nationwide Children's Hospital, Abigail Wexner Research Institute, Clinical and Translational Intramural Funding Program IFPAWRI022023***
- ***Nationwide Children's Hospital, Abigail Wexner Research Institute, Oral-GI Microbiology Research Affinity Group***

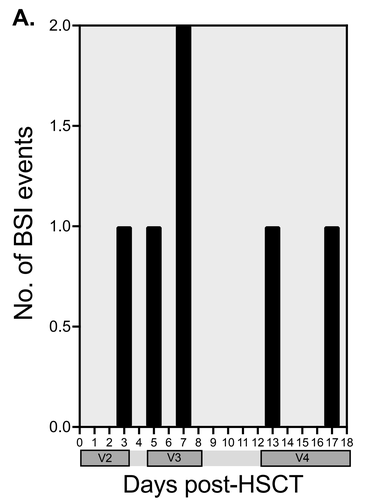
RESULTS

Site-specific microbial community changed based on oral mucositis score

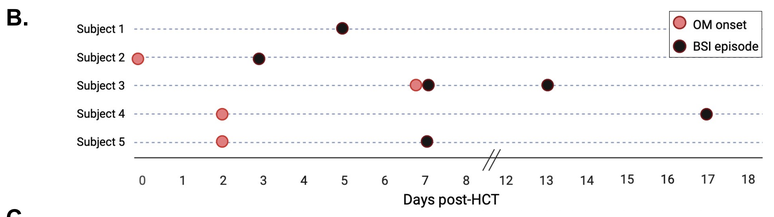


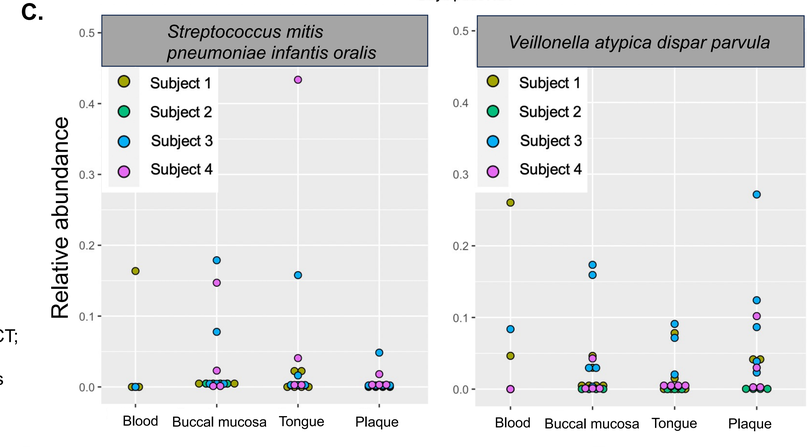
Oral samples (plaque, buccal mucosa, tongue swabs) from all subjects and blood from BSI-positive subjects were collected for 16S rRNA sequencing. Non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity index was used to compare beta-diversity and PERMANOVA test was used to analyze differences in bacterial community composition among samples by **(A)** sampling site or **(B)** oral mucositis grade.

Microbial organisms potentially derived from the oral cavity identified in (+) BSI subjects



A) Number of BSI events observed in our pilot study. V2: Day -1 to +3 post-HCT; V3: Day +5 to +8 post-HCT; V4: After engraftment/before discharge.
B) Timeline displaying onset of OM and BSI episodes in select (+) BSI subjects.
C) Blood microbial profile of select (+) BSI subjects. 16S rRNA sequencing was performed in blood specimens collected from (+) BSI subjects and their corresponding oral samples. For high-resolution analysis, the DADA2 algorithm was used to generate amplicon sequence variants (ASVs) that were taxonomically classified both by the SILVA database within DADA2 and by BLAST alignment to the OSU CORE database. Relative abundance of 16S ASVs of microbial species in blood and corresponding oral niches shown.
D) Patient clinical data. **E)** Absolute neutrophil counts (ANC) of subjects over the course of HCT.





D.

Age	Sex	SCT	Donor	Disease	Conditioning	Oral mucositis	BSI
1	F	PBSCT	Autologous	Medulloblastoma	CB+TT	N	N
1	M	PBSCT	Autologous	Neuroblastoma	CB+TT	Y	Y
2	F	PBSCT	Autologous	Neuroblastoma	TT+CY	Y	N
2	M	PBSCT	Autologous	Neuroblastoma	TT+CY	Y	Y
3	F	PBSCT	Autologous	Neuroblastoma	TT+CY	Y	N
4	M	PBSCT	Autologous	Medulloblastoma	CB+TT	Y	N
6	F	BMT	Sibling	Acute myeloid leukemia	TBI+CY	Y	Y
12	F	PBSCT	Unrelated	*STAT3* GOF	FLU+TRE+ATG+TT+R	Y	N
14	F	BMT	Autologous	Acute lymph. leukemia	ATG+CY+TBI+R	Y	Y
16	M	BMT	Sibling	Acute myeloid leukemia	TBI+CY	Y	Y

CB, carboplatin; TT, thiotepa; CY, cytoxan; TBI, total body irradiation; FLU, fludarabine; TRE, treosulfan; ATG, antithymocyte globulin; R, rituximab.

E.