Supplementary Material

Title: Impact of Gut Colonization with Butyrate Producing Microbiota on Respiratory Viral Infection Following Allo-HCT

Running title: Butyrate-producing bacteria in allo-HCT

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Defining lower respiratory tract infection (LRTI)

There is no formal validated approach to defining combined clinical and radiographic endpoints for lung injury in these complex HCT patients.[1] Presenting signs, symptoms and radiographic findings associated with pulmonary processes are highly variable, due in large part to heterogeneous inflammatory responses during pre-and peri-engraftment periods, as well as a of myriad comorbidities. For this study, the primary endpoint, LRTI, was employed as a broad clinical-radiographic definition. It required a clinical indication for chest imaging (respiratory symptoms, e.g. cough, dyspnea, and/or abnormal vital signs, e.g. fever, hypoxia) and the development of a radiographic parenchymal abnormality that correlated clinically with an infection and influenced management. The operational procedure involved independent chart review by two study physicians (B.W. Haak and F. Adhi) on each of the 360 included study subjects. First, this entailed manual extraction of all respiratory viral infections in the first 180 days following allogeneic hematopoietic stem cell transplant (allo-HSCT). Next, of the patients who were identified with a respiratory viral infection, all diagnostic chest images seven days prior to and 14 days following respiratory virus infection were reviewed, as described by the clinical radiologist in the electronic medical record (EMR). When a study subject was identified as having any new parenchymal abnormality on Computed Tomography (CT) scan or x-ray, information regarding indication for imaging, radiographic patterns, subsequent diagnostic work-up, and therapeutic management were reviewed. Abnormal parenchymal findings were only deemed to be a LRTI if the transplant physician caring for the patient deemed an infection was present, and elicited a treatment plan based on these findings and the clinical picture. Specific diagnoses were assigned to LRTI events only if supportive diagnostic results corroborated with the transplant provider's clinical impression, as documented in the EMR. Abnormal parenchymal findings thought to be caused by volume overload or cardiogenic pulmonary edema (as defined by radiographic appearance, supportive echocardiogram and/or BNP data, and stated as such in the EMR) were excluded when considered isolated explanations for the presenting respiratory signs or symptoms. An impression of atelectasis by the clinical radiologist was also excluded if considered incidental (e.g. fever work-up but no respiratory complaints, with subsequent identification of an extra-pulmonary fever source); however, an infiltrate initially identified as atelectasis in a patient with hypoxia that clearly evolved into pneumonia on repeat imaging was considered a LRTI. A pulmonary specialist (A.I. Geyer) adjudicated a detailed review of the clinical data and provider notes surrounding each event.

Selection criteria of bacteria with known butyrate-producing properties

To verify the findings that bacteria with butyrate-producing properties were associated with protection against LRTI progression following URTI, we selected a group of bacteria with known butyrate-producing properties, adapted from a study that analyzed butyrate-producing pathways in 3,184 sequenced bacterial genomes, and 15 healthy volunteers.[2] This recent study identified a total of 225 bacteria with butyrate-producing properties, of which 61 bacterial species were isolated out of human samples. 44 out of these 61 bacterial species were detected in this cohort (table S3).

REFERENCES

1. Harris B, Morjaria SM, Littmann ER, et al. Gut Microbiota Predict Pulmonary Infiltrates after Allogeneic Hematopoietic Cell Transplantation. Am J Respir Crit Care Med. 2016;194(4):450–63
2. Vital M, Howe AC, Tiedje JM. Revealing the Bacterial Butyrate Synthesis Pathways by Analyzing (Meta)genomic Data. MBio.2014; 5(2):e00889.

Table S1. List of bacterial taxa with known butyrate-producing properties that were selected for analysis

Superkingdom	Phylum	Class	Order	Family	Genus/Species	# samples containing 16S sequences
Bacteria	Actinobacteria	Actinobacteria	Propionibacteriales	Propionibacteriaceae	Propionibacterium acidifaciens	39 (10.8%)
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Odoribacter splanchnicus	0 (0.0%)
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas asaccharolytica	2 (0.6%)
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas endodontalis	4 (1.1%)
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas gingivalis	3 (0.8%)
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas uenonis	0 (0.0%)
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Alistipes putredinis	42 (11.7%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium botulinum	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium butyricum	15 (4.2%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium perfringens	18 (5.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium sporogenes	11 (3.1%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Anaerofustis stercorihominis	29 (8.1%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium cellulosolvens	2 (0.6%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium desmolans	47 (13.1%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium hallii	41 (11.4%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium limosum	36 (10.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium rectale	57 (15.8%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium saphenum	4 (1.1%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Eubacterium ventriosum	14 (3.9%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Pseudoramibacter alactolyticus	26 (7.2%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Anaerostipes caccae	55 (15.3%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio crossotus	13 (3.6%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio fibrisolvens	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Butyrivibrio proteoclasticus	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium saccharolyticum	19 (5.3%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Clostridium symbiosum	48 (13.3%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus catus	35 (9.7%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus comes	62 (17.2%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus eutactus	13 (3.6%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnoanaerobaculum saburreum	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia hominis	13 (3.6%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia intestinalis	24 (6.7%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia inulinivorans	20 (5.6%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Shuttleworthia satelles	57 (15.8%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Anaerococcus hydrogenalis	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Anaerococcus lactolyticus	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Anaerococcus prevotii	10 (2.8%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Anaerococcus tetradius	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Anaerococcus vaginalis	20 (5.6%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Peptoniphilus duerdenii	1 (0.3%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Peptoniphilus harei	0 (0.0%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptoniphilaceae	Peptoniphilus lacrimalis	7 (1.9%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Eubacterium yurii	1 (0.3%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptoclostridium difficile	52 (14.4%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Anaerotruncus colihominis	44 (12.2%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Faecalibacterium prausnitzii	61 (16.9%)
Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Subdoligranulum variabile	96 (26.7%)
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Eubacterium dolichum	54 (15.0%)
Bacteria	Firmicutes	Erysipelotrichia	Erysipelotrichales	Erysipelotrichaceae	Holdemanella biformis	40 (11.1%)
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Acidaminococcaceae	Acidaminococcus fermentans	0 (0.0%)
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Acetonema longum	1 (0.3%)
Bacteria	Firmicutes	Negativicutes	Selenomonadales	Veillonellaceae	Megasphaera micronuciformis	72 (20.0%)
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium gonidiaformans	0 (0.0%)
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium mortiferum	4 (1.1%)
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium nucleatum	54 (15.0%)
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium ulcerans	0 (0.0%)
Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium varium	16 (4.4%)
Bacteria	Spirochaetes	Spirochaetia	Brachyspirales	Brachyspiraceae	Brachyspira murdochii	0 (0.0%)
Bacteria	Spirochaetes	Spirochaetia	Brachyspirales	Brachyspiraceae	Brachyspira pilosicoli	0 (0.0%)
Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema phagedenis	0 (0.0%)
Bacteria	Spirochaetes	Spirochaetia	Spirochaetales	Spirochaetaceae	Treponema vincentii	0 (0.0%)

Total 61 Species, 44 detected in data

Table S2: Case Mix of Allo-HCT recipients not included in the study cohort

Variable	Value	Patients not in cohort	Study cohort	Fisher P-value
Age (years)	≤29	35 (6.3%)	26 (7.2%)	0.741
	30-39	60 (10.8%)	48 (13.3%)	
	40-49	88 (15.9%)	53 (14.7%)	
	50-59	163 (29.5%)	107 (29.7%)	
	≥60	207 (37.4%)	126 (35.0%)	
Sex	Female	224 (40.5%)	131 (36.4%)	0.238
	Male	329 (59.5%)	229 (63.6%)	
Underlying disease	Leukemia	284 (51.4%)	187 (51.9%)	0.766
	Lymphoma	105 (19.0%)	66 (18.3%)	
	Multiple Myeloma	47 (8.5%)	36 (10.0%)	
	Myelodysplastic Syndrome	88 (15.9%)	58 (16.1%)	
	Other Disorders	29 (5.2%)	13 (3.6%)	
Conditioning Intensity	Myeloablative	259 (46.8%)	174 (48.3%)	0.760
	Reduced Intensity	245 (44.3%)	159 (44.2%)	
	Nonmyeloablative	49 (8.9%)	27 (7.5%)	
Stem cell source	Related Identical	81 (14.6%)	74 (20.6%)	0.077
	Unrelated Identical	76 (13.7%)	52 (14.4%)	
	Non-Identical	147 (26.6%)	96 (26.7%)	
	Umbilical Cord	249 (45.0%)	138 (38.3%)	
Time to engraftment	<14 days	393 (71.1%)	270 (75.0%)	0.198
	≥14 days	160 (28.9%)	90 (25.0%)	
Corticosteroid use	No	265 (47.9%)	168 (46.7%)	0.735
	Yes	288 (52.1%)	192 (53.3%)	
Beta.Lactam	No	131 (23.7%)	71 (19.7%)	0.166
	Yes	422 (76.3%)	289 (80.3%)	
Fluoroquinolones	No	101 (18.3%)	62 (17.2%)	0.724
	Yes	452 (81.7%)	298 (82.8%)	
Vancomycin (IV)	No	30 (5.4%)	11 (3.1%)	0.103
	Yes	523 (94.6%)	349 (96.9%)	
Metronidazole	No	464 (83.9%)	300 (83.3%)	0.855
	Yes	89 (16.1%)	60 (16.7%)	
Total		553 (100%)	360 (100%)	-

IV: intravenous

Table S3. Characteristics of Diagnosed Viral Lower Respiratory Tract Infections (47 cases)

Viral LRTI pathogen	Time (post-HCT)	Diagnostic Specimen Type	Butyrate Abundance Group	Butyrate Abundance (16S)	Butyrate (mM)
FluB	125	NP,BAL	Low	0.00068	not performed
Adeno	20	NP,BW,BAL	Absent	0	0.025
Adeno	160	BAL	Low	0.00027	<0.010
Corona(NL63)	140	NP,BAL,BW	Absent	0	<0.010
Adeno	23	NP,BAL,BW	Absent	0.000057	<0.010
Rhino	159	NP,BAL	Low	0.00063	3.7
Rhino	34	NP,BAL	Absent	0	<0.010
RSV	66	NP,BAL	Absent	0	0.031
Metapneumo	30	NP,BW,BAL	Low	0.00048	<0.010
Paraflu3	160	NP,BW	Low	0.00015	<0.010
Corona(OC43)	156	NP,sputum,BW	Absent	0	1.6
Rhino	92	NP,sputum	Low	0.0013	0.1
Rhino	134	NP	Absent	0	0.026
Corona(229E),FluA,RSV	152	NP	Absent	0	0.033
Corona(NL63)	56	NP	Low	0.00033	0.4
Rhino	144	NP	Absent	0.0000074	<0.010
Rhino	117	NP	Absent	0	<0.010
Rhino	40	NP	Absent	0	0.09
Rhino	93	NP	Absent	0	<0.010
Rhino	123	NP	Low	0.00021	0.23
Corona(HKU1)	52	NP	Absent	0	<0.010
Metapneumo	138	NP	Low	0.0024	<0.010
Rhino	95	NP	Low	0.00078	<0.010
Paraflu1	34	NP	Absent	0	<0.010
Paraflu3	130	NP	Low	0.00033	<0.010
Rhino	167	NP	Low	0.00016	0.04
Rhino	80	NP	Low	0.0018	<0.010
Rhino	67	NP	Low	0.00073	<0.010
Adeno,Metapneumo,Rhino	151	NP	High	0.099	0.15
Rhino	123	NP	Absent	0	2.6
Metapneumo	90	NP	Low	0.0011	<0.010
Corona(OC43)	17	NP	Absent	0	<0.010
Rhino	89	NP	Absent	0	0.025
Corona(OC43),RSV	55	NP	Absent	0	0.32
RSV	75	NP	Absent	0	0.03
Rhino	62	NP	Absent	0	0.92
Corona(NL63)	96	NP	Absent	0	0.028
Corona(OC43),Rhino	104	NP	Low	0.002	<0.010
RSV	61	NP	Low	0.00085	<0.010
Paraflu4,RSV	136	NP	Absent	0	0.35
Rhino	17	NP	Low	0.00057	0.031
Corona(OC43)	37	NP	Absent	0.000034	<0.010
Metapneumo	81	NP	Low	0.00054	<0.010
Rhino	110	NP	Low	0.002	<0.010
RSV	94	NP	Low	0.0024	<0.010
Corona(229E),Metapneumo,RSV	160	NP	High	0.048	<0.010
RSV	136	NP	Low	0.0056	<0.010

NP: nasopharynx swab; BAL: bronchoalveolar lavage; BW: bronchial wash; Sputum: induced sputum; Adeno: Adenovirus; Rhino: Rhinovirus; Corona: Coronavirus; RSV: Respriatory Synctial Virus; Flu: Influenza; Paraflu: Parainfluenza; Metapneumo: Human Metapneumovirus

Table S4: Analysis of alternative microbiota predictors of viral LRTI

Microbiota variable	Hazard ratio	P-value
Lachnospiraceae abundance (≥0·001)	0.97 (0.55 - 1.72)	0.930
Ruminococcaceae abundance (≥0·001)	0.79 (0.40 - 1.47)	0.475
Bacteroidetes abundance (≥0·001)	0.91 (0.50 - 1.61)	0.749
Microbial diversity (<2)	1.00	–
Microbial diversity (2-4)	1.25 (0.66 - 2.31)	0.481
Microbial diversity (>4)	0.58 (0.23 - 1.30)	0.198