THE LANCET Respiratory Medicine

Supplementary appendix

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Association Between Lung Microbiome and Disease Progression in IPF
Web Extra Material

Supplemental Table 1. 71 subjects were recruited enrolled in COMET-IPF. Below is a table outlining the data that was missing resulting in non-inclusion and the final n=55*.

DLCO	Six minute walk test	History of Gastroesophageal Reflux Disease
X	X	
	X	
	X	
	X	
	X	
		X
X		
	X	
X		
	X	
	X	
	X	
	X	
X	X	
X		
X		

*Of the 15 individuals removed due to missing DLCO or 6MWT data, 3/15 had Streptococcus greater than our specified threshold, 3/15 had Staphylococcus greater than our specified threshold and 1/15 had both such that 7/15 had Streptococcus or Staphylococcus greater than threshold.

Supplemental Table 2. Patient characteristics of subjects not included in the multivariate analysis due to missing data.

Demographics	N=55	N=16	P-value
Age (years)	64·3 (7·5)	67·8 (5·9)	0·06
Male gender	37/55 (67·2%)	11/16 (68·8%)	0·99
Past Smoker	35/55 (63·6%)	12/16 (75%)	0·22
Current Smoker	2/55 (3·64%)	0 (0)	0·99
Pack-years	47·6 (52·3)	26·4 (21·2)	0·06
FEV1% predicted	73·2 (18·3)	71·9 (23·8)	0·86
FVC% predicted	70·1 (17·0)	67.2 (20.6)	0·64
DLCO% predicted	42·3 (14·0)	-	-
Desaturation <88% during 6MWT	29/55 (52·7%)	-	-
History of GERD	31/55 (56·4%)	10/16 (67·7%)	0·52
Mean follow-up time (days)	355·8	361·5 (190·3)	0·92
Microbiome Indices			
Shannon Diversity Index	2·41 (0·46)	2·14 (0·46)	0·05
Inverse Simpson Index	8·52 (3·86)	6·03 (2·62)	0·005

Supplemental Table 3. % Relative Abundance of individual OTU's among subjects where OTU was detected

Rank	Genus Level Taxonomy	OTU name	Number of Subjects occurred	% Relative Abundance per Subject (SD)
1	Prevotella	1327	37	14·1 (12)
2	Veillonella	1331	41	9·1 (7·7)
3	Cronobacter[21]	1283	35	8·8 (9·4)
4	Clostridium	1286	32	9·0 (12·1)
5	Porphyromonas	1325	25	11·0 (9·6)
6	Pseudomonas	1200	26	7·9 (12·9)
7	Flavobacterium	1301	30	6·1 (4·9)
8	Haemophilus	1349	35	4·9 (4·8)
9	Fusobacterium	1328	28	5·5 (5·0)
10	Pseudomonas	1302	23	6·6 (7·5)
11	Streptococcus	1345	14	10·1 (9·7)
12	Bacteroides	1240	20	6·4 (5·5)
13	Prevotella	1260	22	4·6 (3·4)
14	Staphylococcus	1348	26	3·8 (4·3)
15	Neisseria	1314	11	8·7 (7·8)
16	Halomonas	1277	29	2·9 (1·9)
17	Enterococcus	1352	19	4·1 (11·8)
18	Streptococcus	1350	11	6·8 (4·5)
19	Gemella	1336	23	2·9 (3·2)
20	Prevotella	1291	18	3·2 (2·9)
21	Bacteroides	1290	19	3·0 (2·8)
22	Pseudomonas	1274	18	3·1 (2·5)
23	Propionibacterium	1305	6	9·0 (9·5)
24	Prevotella	1261	21	2·6 (2·5)
25	Bordetella	1278	12	4·1 (3·2)
26	Flavobacterium	1146	13	3·4 (2·9)
27	Oribacterium	1273	18	2·5 (1·8)
28	Tropheryma	1110	3	13·8 (9·7)

29	Chryseobacterium	1165	11	3·5 (3·0)
30	Mucilaginibacter	1205	1	34·7 (0)
31	Actinomyces	1254	20	1·7(1·3)
32	Sphingomonas	1058	9	3·0 (2·7)
33	Microbacterium	1160	14	1·7 (1·5)
34	Prevotella	1333	4	5·6 (5·8)
35	Burkholderia	1299	8	2·6 (2·5)
36	Megasphaera	1224	14	1·4 (0·7)
37	Lactobacillus	1143	7	2·8 (2·6)
38	Capnocytophaga	1117	7	2·8 (3·3)
39	Arcicella	0551	5	3·7 (2·6)
40	Leptotrichia	1256	9	1·9 (1·0)
41	Turicella	1156	13	1·3 (0·6)
42	Stenotrophomonas	1036	8	2·0 (1·1)
43	Oscillibacter	0969	3	5·2 (3·5)
44	Prevotella	1341	4	3·7 (2·2)
45	Ralstonia	1157	8	1·8 (1·0)
46	Burkholderia	1294	4	3·5 (4·3)
47	Capnocytophaga	1051	6	2·2 (1·1)
48	Prevotella	1337	7	1·7 (1·7)
49	Prevotella	1289	1	11·3 (0)
50	Prevotella	1196	4	2·8 (3·2)

Supplemental Table 4. Adjusted Cox regression model to determine the association of pulmonary IPF microbiome to disease progression defined as death, acute exacerbation, lung transplant, or decline in FVC of 10% or DLCO of 15%. This model is identical to that demonstrated in Table 2C, additionally adjusted for clinical center of recruitment.

Variable	Relative Risk	95% Confidence Interval	P-value
Age (per 10 years)	0·78	0·40, 1·53	0·48
Male gender	0·51	0·17, 1·54	0·23
Ever smoker	1·23	0·50, 3·01	0·65
FVC (per 10%)	1·28	0·90, 1·82	0·17
DLCO (per 10%)	1·22	0·68, 2·16	0·50
Desaturation <88%	9·94	2·39, 4·32	0·002
Gastroesophageal reflux	10·48	2·72, 40·39	0·0006
Staph threshold	15·26	3·95, 58·92	<0·0001
Strep threshold	26·46	6·40, 109·33	<0·0001
Shannon Index	0·47	0·26, 0·82	0·008
Concordance Index	0·82		

Supplemental Table 5. Adjusted Cox regression model to determine the association of pulmonary IPF microbiome to disease progression defined as death, acute exacerbation, lung transplant, or decline in FVC of 10% or DLCO of 15%. This model includes the same covariates as Table 2, but a weighted Cox regression model has been used to better account for the possibility of non-proportional hazards.

Supplemental Table 4A.			
Variable	Relative Risk	95% Confidence Interval	P-value
Age (per 10 years)	0·76	0·35, 1·67	0·50
Male	0·60	0·22, 1·64	0·32
Ever Smoker	2·44	1·02, 5·85	0·05
FVC (per 10%)	1·26	0·87, 1·83	0·22
DLCO (per 10%)	0·86	0·40, 1·86	0·70
Desaturation <88%	3·67	1·27, 10·62	0·02*
Gastroesophageal reflux	3·24	0·90, 11·62	0·07
Principal Component 1	1·83	1·31, 2·54	0·0003*
Principal Component 2	1·70	1·11, 2·60	0·01*
Shannon Diversity Index	0·71	0·43, 1·16	0·17

Supplemental Table 4B.			
Variable	Relative Risk	95% Confidence Interval	P-value
Age (per 10 years)	0·75	0·34, 1·65	0·47
Male	0·61	0·22, 1·68	0·34
Ever Smoker	2·41	1·01, 5·78	0·05
FVC (per 10%)	1·27	0·87, 1·84	0·22
DLCO (per 10%)	0·86	0·40, 1·86	0·70
Desaturation <88%	3·79	1·30, 11·01	0·01*
Gastroesophageal reflux	3·24	0·90, 11·64	0·07
Streptococcus OTU 1345 % relative abundance	1·99	1·44, 2·76	<0·0001*
Staphylococcus OTU 1348 % relative abundance	1·64	1·06, 2·55	0·03*
Shannon Diversity Index	0·72	0·44, 1·18	0·20

Supplemental Table 4C.			
Variable	Relative Risk	95% Confidence Interval	P-value
Age (per 10 years)	0·68	0·30, 1·54	0·36
Male	0·72	0·21, 2·43	0·60
Ever Smoker	1·73	0·77, 3·89	0·18
FVC (per 10%)	1·29	0·87, 1·90	0·20
DLCO (per 10%)	0·97	0·49, 1·93	0·94
Desaturation <88%	5·86	1·78, 19·25	0·004*
Gastroesophageal reflux	3·67	1·25, 10·89	0·02*
Streptococcus OTU 1345 >threshold (3.9% relative abundance)	9·57	2·92, 31·30	0·0002*
Staphylococcus OTU 1348 >threshold (1.8% relative abundance)	5·00	1·96, 12·77	0·0008*
Shannon Diversity Index	0·55	0·35, 0·89	0·014*

Supplemental Table 6. Distribution of event types that were included in the composite endpoint experienced by individuals in the study.

Event type	N=36
Death	1
Lung transplant	1
Acute Exacerbation	2
Decline in DLCO	16
Decline in FVC	11
Decline in DLCO and FVC	5

Supplemental Table 7. Demographics based on presence or absence of *Streptococcus* OTU 1345 and *Staphylococcus* OTU 1348 above defined thresholds.

Demographics	*Streptococcus* OTU 1345 (n=8)	No *Streptococcus* OTU 1345 (n=47)	P-value	*Staphylococcus* OTU 1348 (n=16)	No *Staphylococcus* OTU 1348 (n=39)	P-value	Total
Age	68·9 (7.46)	64 (7·3)	0·06	62 (7·9)	65·2 (7·3)	0·16	64·3 (7·5)
Male	5/8 (62·5%)	32/47 (68·1%)	0·76	8/16 (50%)	29/39 (74·4%)	0·15	37 (67·2%)
Current Smoker	5/8 (62·5%)	30/47 (63·8%)	0·94	11/16 (68·8%)	24/39 (61·5%)	0·84	35 (63·6%)
Past Smoker	0 (0%)	2/47 (4·26%)	0·55	0 (0%)	2/39 (5·13%)	0·90	2(3·64%)
Pack-years	28·6 (13·4)	51 (55·8)	0·56	40 (23·8)	50·6 (60·3)	0·60	47·6 (52·3)
FEV1% predicted	73·8 (15·4)	73·1 (18·8)	0·92	72·4 (18·5)	73·5 (18·4)	0·83	73·2 (18·3)
FVC%	67·2 (12)	70·6 (17·7)	0·60	71·6 (18·1)	69·5 (16·7)	0·68	70·1 (17·0)
DLCO%	38·6 (13·4)	42·9 (14·0)	0·43	41·1 (15·0)	42. · (13·8)	0·70	42·3 (14·0)
Desaturation <88%	4/8 (50%)	25/47 (53·2%)	0·87	7/16 (43·7%)	22/39 (56·4%)	0·58	29 (52·7%)
History of Reflux	7/8 (87·5%)	24/47 (51·1%)	0·12	6/16 (37·5%)	25/39 (64·1%)	0·13	31 (56·4%)
Microbiome							
SDI	2·53 (0·34)	2·39 (0·47)	0·42	2·62 (0·31)	2·33 (0·48)	0·03	2·41 (0·46)
Inv. Simpson	9·08 (2·44)	8·47 (4·06)	0·68	10·78 (4·25)	7·65 (3·33)	0·005	8·52(3·86)

Supplemental Table 8. % Relative abundance of individual OTU's for the two IPF lung explants demonstrated in Figure 2.

	IPF 1 Proximal Brush	IPF 1 Distal Brush	IPF 2 Proximal Brush	IPF 2 Distal Brush
Pseudomonas OTU1302			82.19	58.12
Streptococcus OTU1350	100	23.89		
Propionibacterium		62.51	2.89	
Blautia				17.67
Streptococcus OTU 1348		0.81		15.29
Pseudochrobactrum				8.92
Corynebacterium		6.64		
Anaerofilum			5.71	
Unclassified			5.56	
Haemophilus		4.29		
Staphylococcus OTU1348		1.86		
Serratia			1.45	
Pantoea			1.14	
Microbacterium			1.07	