Online Supplement

Common Respiratory Pathogens Other Than Haemophilus in Small Airways Are Associated With Neutrophilic Inflammation and Poor Health Status in Stable COPD Patients

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Table 1: Pathogens detected for each subject who tested positive in the study (for Prevotella and Veillonella species coverage kindly refer to table 2).

Subject	Pathogen 1	pathogen 2	Pathogen 3	Pathogen 4
1	Moraxella catarrhalis	Streptococcus pneumoniae		
2	Moraxella catarrhalis	streptococcus pneumoniae		
3	Moraxella catarrhalis			
4	Rhinovirus			
5	Haemophilus influenzae	Veillonella species		
6	Haemophilus influenzae	Streptococcus pneumoniae		
7	Haemophilus influenzae	Rhinovirus	Streptococcus pneumoniae	Veillonella species
8	Haemophilus influenzae	Prevotella species		
9	Haemophilus influenzae	Prevotella species		
10	Haemophilus influenzae	Moraxella catarrhalis	Streptococcus pneumoniae	
11	Haemophilus influenzae			
12	Haemophilus influenzae			
13	Haemophilus influenzae			
14	Haemophilus influenzae			
15	Haemophilus influenzae			
16	Haemophilus influenzae			
17	Haemophilus influenzae			
18	Streptococcus pneumoniae			

Table 2: Species coverage on the Randox cystic fibrosis respiratory pathogen array.

Burkholderia cepacia complex	**Streptococcus species**	**Non-tuberculous mycobacterium species**	**Pandoraea species**	**Prevotella species**
B. ambifaria	S. agalactiae	M. gordonae	P. apista	P. bivia
B. anthina	S. anginosus	M. kansasii	P. norimbergensis	P. buccae
B. arboris	S. australis	M. malmoense	P. pulmonicola	P. denticola
B. cenocepacia	S. constellatus	M. marseillense	P. sputorum	P. disiens
B. cepacia	S. cristatus	M. parascrofulaceum	P. pnomenusa	P. histicola
B. contaminans	S. dysgalactiae	M. scrofulaceum	**Scedosporium species**	P. intermedia
B. diffusa	S. gordonii	M. simiae	S. apiospermum	P. melaninogenica
B. dolosa	S. infantis	M. szulgai	S. aurantiacum	P. multiformis
		Mycobacterium abscessus subgroup		
B. gladioli	S. intermedius		S. dehoogii	P. nigrescens
B. lata	S. massiliensis	M. abscessus	S. prolificans	P. oralis
B. latens	S. mitis	M. bolletii	S. pseudallescheria boydii	P. oris
B. mallei	S. mutans	M. chelonae	S. pseudallescheria minutispora	P. oulorum
B. metallica	S. oralis	M. massiliense	S. pseudallescheria ellipsoidea	P. pallens
		Mycobacterium avium complex	**Ralstonia species**	
B. multivorans	S. parasanguinis			P. salivae
B. oklahomensis	S. peroris	M. avium	R. gilardii	P. tannerae
B. pseudomallei	S. pneumoniae	M. chimaera	R. insidiosa	P. veroralis
B. pyrrocinia	S. pseudopneumoniae	M. intracellulare	R. mannitolilytica	**Veillonella species**
B. seminalis	S. salivarius	M. paratuberculosis	R. pickettii	V. dispar
B. stabilis	S. sanguinis		R. respiraculi	V. parvula
B. ubonensis	S. thermophilus		R. taiwanensis	V. atypica
B. vietnamiensis	S. vestibularis			

Table 3: Multiple linear regression to control for variables affecting bronchoalveolar lavage neutrophil%.

Predictor	B (95% CI)	Standard Error of B	Beta	Tolerance	Variance inflation factor (VIF)	P-value
Step 1						
Intercept	66.2 (-33.7 to 166.1)	48.9				0.2
FEV_1% of Predicted	-0.7 (-1.2 to -0.1)	0.3	-0.4	**0.9**	**1**	**0.014**
Smoking	-3.2 (-22.3 to 16)	9.4	-0.06	0.7	1.3	0.7
Age	0.04 (-1.2 to 1.3)	0.6	0.01	0.7	1.4	0.9
Sex	-8.9 (-34.4 to 16.5)	12.5	-0.01	0.9	1	0.5
Step 2						
Intercept	11.9 (-82 to 105.9)	45.9				0.8
FEV_1% of Predicted	-0.6 (-1.1 to -0.1)	0.2	-0.4	**0.9**	**1**	**0.014**
Smoking	1.2 (-15.8 to 18.1)	8.3	0.02	0.7	1.4	0.9
Age	0.6 (-0.6 to 1.7)	0.6	0.2	0.6	1.6	0.3
Sex	-2.7 (-25.3 to 20)	11.1	-0.03	0.9	1	0.8
Pathogen-positive*	25.3 (9.2 to 41.4)	7.9	0.5	**0.9**	**1.1**	**0.003**

*Subjects who had a positive result for only Haemophilus influenzae were merged with subjects who had no pathogens detected.

Table 4: Multiple linear regression model fit statistics.

Model Summary[c]				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.46[a]	0.21	0.10	23.4
2	0.65[b]	0.42	0.31	20.4
a. Predictors: (Constant), Sex, Smoking, FEV_1, Age				
b. Predictors: (Constant), Sex, Smoking, FEV_1, Age, Pathogen-positive*				
c. Dependent Variable: BAL neutrophil%				

*Subjects who had a positive result for only Haemophilus influenzae were merged with subjects who had no pathogens detected.

Quality control and DNA concentration:

Each sample after nucleic acid extraction was tested for DNA concentration via NanoDrop ND-8000 spectrophotometer (ThermoFisher Scientific, Waltham, MA) and results are shown in Table 5. We did not find an association between pathogen positivity and DNA concentration (P=0.4) or BAL return volume (P=0.7).

Table 5: BAL return volume and DNA concentration for each sample tested.

Subject	BAL return volume (ml)	DNA Concentration (ng/uL)
1	30	52
2	30	49
3	33	66

4	22.1	51
5	22.2	56
6	15	60
7	20	78
8	28	82
9	30	54
10	25	72
11	25	67
12	10	29
13	27.5	67
14	29	47
15	27	329
16	25	49
17	40	48
18	25	67
19	30	46
20	30	55
21	26.5	64
22	30	69
23	40	44
24	30	46
25	30	58
26	30	75
27	30	65
28	30	41
29	25	50
30	30	91
31	30	62
32	30	57
33	28	56
34	30	67

35	30	53
36	30	64
37	27	51
38	32	66

Table 6: BAL cell differential results (Pathogen positive included subjects who tested positive for only Haemophilus influenzae).

BAL Cell type %	Pathogen negative [median, IQR]	Pathogen Positive [median, IQR]	P value (Mann-Whitney U test)
Neutrophils	4 (2-16.2)	20 (5.6-45.2)	0.024
Macrophages	86.7 (79-91.7)	65.5 (38.5-90)	0.048
Eosinophils	1.2 (0-2.7)	1 (0.3-2.2)	0.7
Lymphocytes	3.5 (2-8.3)	3.5 (2-11.5)	0.6

Table 7: BAL cell differential results (Pathogen negative included subjects who tested positive for only Haemophilus influenzae).

BAL Cell type %	Pathogen negative [median, IQR]	Pathogen Positive [median, IQR]	P value (Mann-Whitney U test)
Neutrophils	5 (3-17)	40 (8-62)	0.013
Macrophages	86 (75-92)	56 (34-90)	0.041
Eosinophils	1 (0-3)	1 (0.7-2)	0.8
Lymphocytes	4 (2-9.5)	3 (1-8)	0.3