Antimicrobial peptides, disease severity and exacerbations in Bronchiectasis

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ONLINE DATA SUPPLEMENT

METHODS

Air-liquid interface nasal epithelial cell culture

For the nasal cilia stuff, healthy controls were recruited from NHS Tayside and University of Dundee staff aged 25 to 60 years old. All controls had no history of rhinitis, asthma or other respiratory disease and were required to be free of upper respiratory tract infection for at least 6 weeks prior to nasal sampling.

RESULTS

AMP levels and FACED score

High sputum LL-37 levels were detected in patient with severe FACED score (7633 (719.1-21135) vs 2678 (30.1-9056) vs 329.6 (21.8-6412) ng/ml, p=0.05) while sputum SLPI levels were the lowest in the most severe group (236.2 (84.7-901.5) vs 769.7 (125.7-3592) vs 637.4 (296.0-3812) ng/ml, p=0.1) (**Figure E1).**

Table E1. Patient demographics, clinical characteristics and prior treatments among non-frequent exacerbators and frequent exacerbators.

	Non-frequent exacerbators (n = 67)	Frequent exacerbators (n = 68)	P value
Age (mean ± SD)	69.3 (±10.6)	68.7 (±10.5)	0.3
Male	32 (54.2)	27 (45.8)	0.3
Smoking status			
Never	44 (65.7)	38 (55.9)	0.1
Ex-smoker	13 (19.4)	10 (14.7)	
Current	10 (14.9)	20 (29.4)	
Comorbidities			
Cardiovascular disease	14 (20.9)	18 (26.5)	0.4
Diabetes mellitus	6 (9.0)	7 (10.3)	0.8
Stroke	5 (7.5)	7 (10.3)	0.6
Treatment			
Inhaled long-acting bronchodilator	35 (52.2)	53 (77.9)	0.002
Inhaled corticosteroids	24 (35.8)	35 (59.3)	0.07
Inhaled antibiotics	2 (4.0)	4 (7.0)	0.5
Chronic macrolide therapy	17 (25.4)	14 (20.6)	0.5
FEV_1 (% pred) (mean ± SD)	87.5 (±27.7)	69.3 (±26.2)	<0.0001
FVC (% pred) (mean ± SD)	101.6 (±26.1)	87.1 (±26.2)	0.004
BMI (kg/m^2) (mean ± SD)	26.4 (±4.4)	25.4 (±6.5)	0.06
Aetiology			
Idiopathic	33 (49.3)	28 (41.2)	0.1
Post-infective	12 (17.9)	10 (14.7)	
Post-TBC	8 (11.9)	4 (5.9)	
Others	14 (20.9)	26 (38.2)	
Exacerbations previous 12 months			
0	24 (35.8)	0 (0.0)	<0.0001
1	28 (41.8)	0 (0.0)	
2	15 (22.4)	0 (0.0)	
3	0 (0.0)	20 (29.4)	
4 or more	0 (0.0)	48 (70.6)	
BSI (mean ± SD)	5.8 (±3.0)	10.3 (±4.3)	<0.0001
FACED (mean ± SD)	2.1 (±1.4)	2.6 (±1.7)	0.1

Data are presented as n(%) unless otherwise indicated.

BMI, body mass index; BSI, Bronchiectasis Severity Index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; PPB, potentially pathogenic bacteria; TBC, tuberculosis; SD, standard deviation.

Table E2. Patient demographics, clinical characteristics and prior treatments among non-infected, infected by *P. aeruginosa* and infected by other pathogens

	Non-infected (*n* = 49)	Infected by other pathogens (*n* = 47)	Infected by *P. aeruginosa* (*n* = 39)	*P* value
Age (mean ± SD)	67.8 (10.6)	70.9 (10.6)	68.7 (10.3)	0.4
Male	17 (34.7)	24 (51.1)	18 (46.2)	0.3
Smoking status				
Never	31 (63.3)	24 (51.1)	27 (69.2)	0.2
Ex-smoker	9 (18.4)	7 (14.9)	7 (17.9)	
Current	9 (18.4)	16 (34.0)	5 (12.8)	
Comorbidities				
Cardiovascular disease	10 (20.4)	11 (23.4)	11 (28.2)	0.7
Diabetes mellitus	6 (12.2)	6 (12.8)	1 (2.6)	0.2
Stroke	4 (8.2)	4 (8.5)	4 (10.3)	0.9
Treatment				
Long-acting bronchodilator	28 (57.1)	27 (57.4)	34 (85)	0.01
Inhaled corticosteroids	15 (30.6)	24 (51.1)	20 (51.3)	0.07
Inhaled antibiotics	0 (0)	4 (8.5)	2 (5)	0.1
Chronic macrolide therapy	12 (24.5)	9 (19.1)	10 (25.6)	0.7
FEV_1 (% pred) (mean ± SD)	85.5 (23.5)	83.6 (29.3)	62.8 (27.3)	<0.001
FVC (% pred) (mean ± SD)	98.1 (23.7)	99.7 (26.2)	83.1 (29.2)	0.008
BMI (kg/m^2) (mean ± SD)	26.3 (5.6)	27.6 (5.7)	23.3 (4.2)	0.001
Bronchiectasis etiology				
Idiopathic	16 (32.7)	29 (61.7)	16 (41.0)	0.1
Post-infective	9 (18.4)	6 (12.8)	7 (17.9)	
Post-tuberculosis	6 (12.2)	1 (2.1)	5 (12.8)	
Others	18 (36.7)	11 (23.4)	11 (28.2)	
Exacerbations previous 12 months				
0	13 (26.5)	9 (19.1)	2 (5.1)	0.1
1	13 (26.5)	8 (17.0)	7 (17.9)	
2	5 (10.2)	4 (8.5)	6 (15.4)	
3	8 (16.3)	6 (12.8)	6 (15.4)	
4 or more	10 (20.4)	20 (42.6)	18 (46.2)	
BSI (mean ± SD)	5.7 (3.2)	7.7 (3.8)	11.5 (4.0)	<0.001
FACED (mean ± SD)	1.7 (1.2)	1.9 (1.1)	3.73 (1.4)	<0.001

Data are presented as n(%) unless otherwise indicated.

BMI, body mass index; BSI, Bronchiectasis Severity Index; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PPB, potentially pathogenic bacteria; SD, standard deviation.

Table E3.- Sputum LL-37, SLPI, lactoferrin and lysozyme levels in bronchiectasis patients with and without inhaled antibiotic treatment, chronic macrolide therapy and inhaled corticosteroids treatment.

	No chronic macrolides n=104 (77%)	**Chronic macrolides** n= 31 (27%)	**p-value**
Sputum LL-37 (ng/ml)	2440.4 (18.4-7422.1)	26.0 (11.4-10667.6)	0.3
Sputum SLPI (ng/ml)	535.2 (191.6-2610.3)	637.4 (89.9-4042.3)	0.6
Sputum Lactoferrin (µg/ml)	118.5 (50.2-248.3)	55.7 (37.0-300.4)	0.2
Sputum Lysozyme (µg/ml)	69.2 (42.1-106.0)	63.7 (37.2-95.2)	0.7

	No ICS n=76 (56%)	**ICS** n= 59 (44%)	**p-value**
Sputum LL-37 (ng/ml)	1986.3 (17.6-6074.7)	361.9 (12.1-12334.0)	0.6
Sputum SLPI (ng/ml)	659.0 (173.4-4220.4)	509.9 (129.0-2569.0)	0.5
Sputum Lactoferrin (µg/ml)	117.5 (49.5-239.8)	113.5 (40.39-285.0)	0.7
Sputum Lysozyme (µg/ml)	68.4 (37.5-103.0)	75.6 (42.5-106.2)	0.9

	No inhaled antibiotic n= 129 (96%)	**Inhaled antibiotic** n=6 (4%)	**p-value**
Sputum LL-37 (ng/ml)	1443.7 (17.0-8108.1)	2654.7 (29.2-9348.6)	0.3
Sputum SLPI (ng/ml)	560.7 (178.5-3534.5)	246.1 (94.7-861.2)	0.8
Sputum Lactoferrin (µg/ml)	114.6 (46.1-246.0)	136.9 (43.3-385.5)	0.6
Sputum Lysozyme (µg/ml)	69.5 (42.3-105.6)	41.6 (19.1-114.4)	0.5

Data are presented as median (Interquartile range). ICS: Inhaled corticosteroids

Table E4.- Analysis of negative binomial model with adjustments.

		Effect estimate	95% CI	p-value
LL-37 ≥1500 ng/ml		**IRR**		
Exacerbation Rate	Unadjusted	1.57	1.04-2.37	0.02
	Adjusted for BSI	1.53	1.01-2.31	0.04
	Adjusted for site	1.52	1.01-2.28	0.04
	Fully adjusted	1.51	0.97-2.34	0.068
		HR		
Time to first exacerbation	Unadjusted	1.68	1.13-2.50	0.01
	Adjusted for BSI	1.58	1.05-2.36	0.02
	Adjusted for site	1.68	1.11-2.56	0.01
	Fully adjusted	1.72	1.12-2.64	0.01
SLPI <1000 ng/ml		**IRR**		
Exacerbation Rate	Unadjusted	0.76	0.50-1.15	0.1
	Adjusted for BSI	0.77	0.50- 1.17	0.2
	Adjusted for site	0.73	0.48- 1.11	0.1
	Fully adjusted	0.63	0.39-0.98	0.04
		HR		
Time to first exacerbation	Unadjusted	0.63	0.42-0.94	0.02
	Adjusted for BSI	0.68	0.45-1.03	0.06
	Adjusted for site	0.70	0.46-1.05	0.08
	Fully adjusted	0.65	0.41-1.02	0.059

Fully adjusted includes adjustment for bronchiectasis severity, gender, aetiology, inhaled corticosteroid use and site.

IRR= incidence rate ratio, HR= hazard ratio, BSI=Bronchiectasis Severity Index

FIGURE LEGENDS

Figure E1. A: Sputum LL-37 levels of FACED score, divided as mild (0-2 points), moderate (2-4 points) and severe (5-7 points), and B: Sputum SLPI levels of FACED score. p values are obtained by Kruskal-Wallis test. Graphs are represented as mean with SEM.

Figure E2. Relationship between sputum AMP levels (A: LL-37, B: lysozyme, C: lactoferrin and D: SLPI) and FEV_1% predicted. Rho and p-value were obtained from Spearman rank correlation.

Figure E3. A: Sputum LL-37 and B: SLPI levels and frequency of exacerbations in the previous year. p values are obtained by Kruskal-Wallis test. Graphs are represented as mean with SEM.

Figure E4. Relationship between sputum AMP levels (A: LL-37, B: lysozyme, C: lactoferrin) and neutrophil elastase activity (Ns=73). Rho and p-value were obtained from Spearman rank correlation.

Figure E5. A: ROC curve of sputum LL-37 for predicting severe exacerbation (Area under the curve (AUC) 0.76, 95% confidence interval (CI) 0.65-0.86, p<0.0001). B: ROC curve of sputum SLPI levels for predicting severe exacerbation (AUC 0.72, 95% CI 0.62-0.86, p<0.0001).

Figure E1:

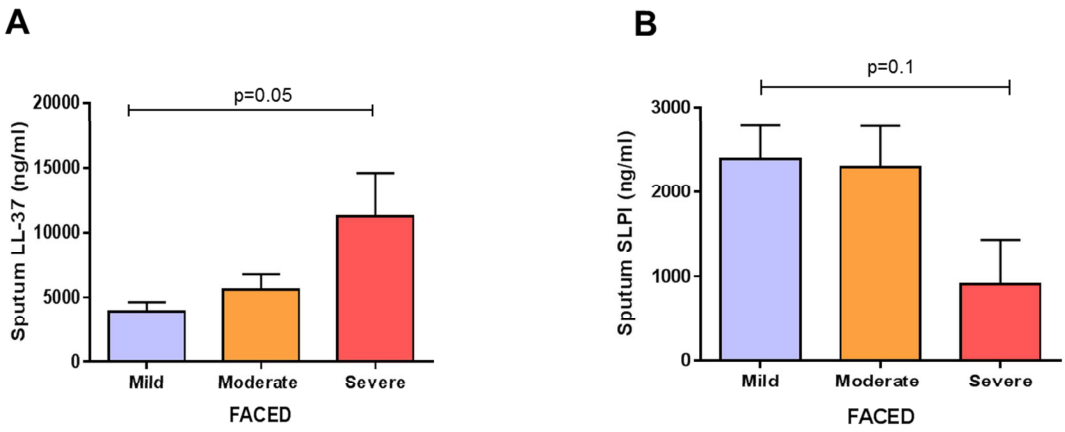

A
p=0.05
Sputum LL-37 (ng/ml)
20000
15000
10000
5000
0
Mild
Moderate
Severe
FACED
B
p=0.1
Sputum SLPI (ng/ml)
3000
2000
1000
0
Mild
Moderate
Severe
FACED

Figure E2:

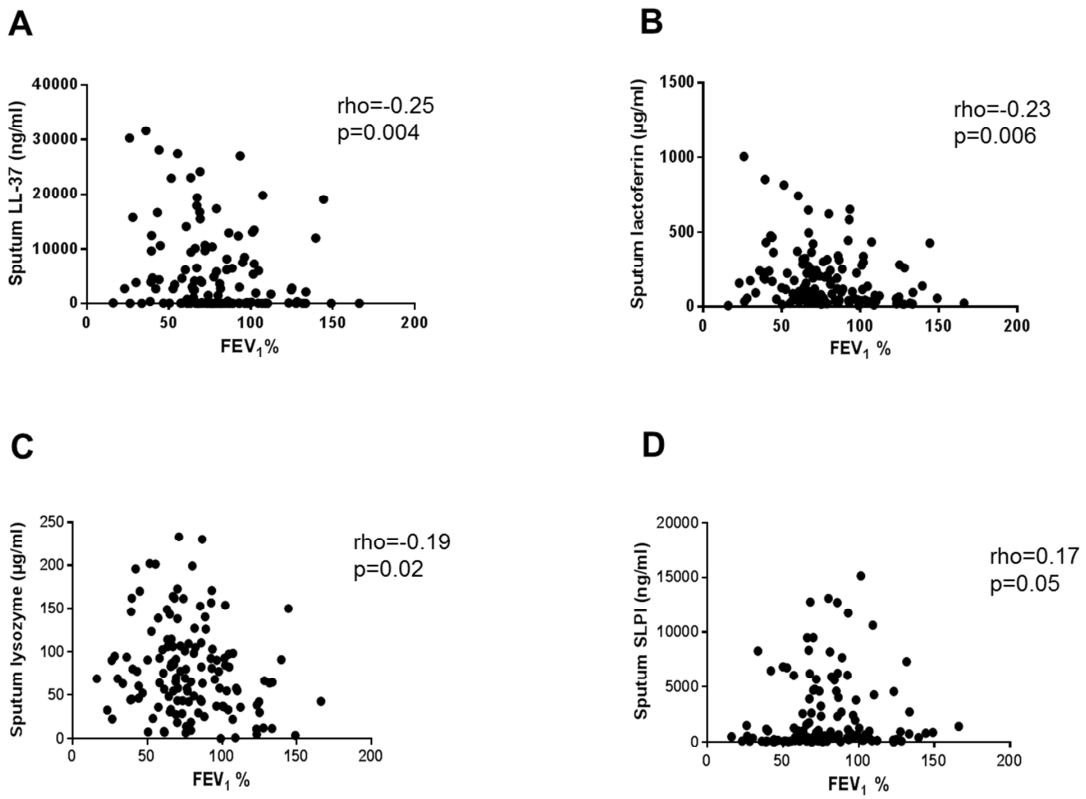

A
Sputum LL-37 (ng/ml)
40000
30000
20000
10000
0
0
50
100
150
200
FEV1%
rho=-0.25
p=0.004
B
Sputum lactoferrin (µg/ml)
1500
1000
500
0
FEV1 %
rho=-0.23
p=0.006
C
Sputum lysozyme (µg/ml)
250
200
150
100
50
FEV1 %
rho=-0.19
p=0.02
D
Sputum SLPI (ng/ml)
20000
15000
10000
5000
FEV1 %
rho=0.17
p=0.05

Figure E3:

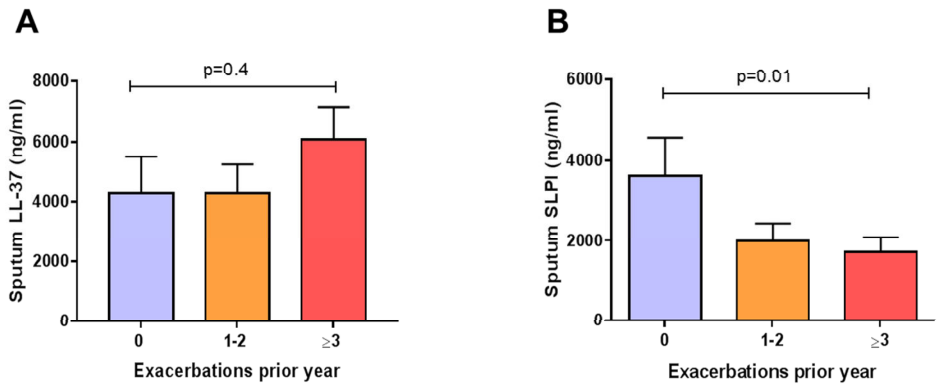

A
p=0.4
Sputum LL-37 (ng/ml)
8000
6000
4000
2000
0
0
1-2
≥3
Exacerbations prior year
B
p=0.01
Sputum SLPI (ng/ml)
6000
4000
2000
0
0
1-2
≥3
Exacerbations prior year

Figure E4:

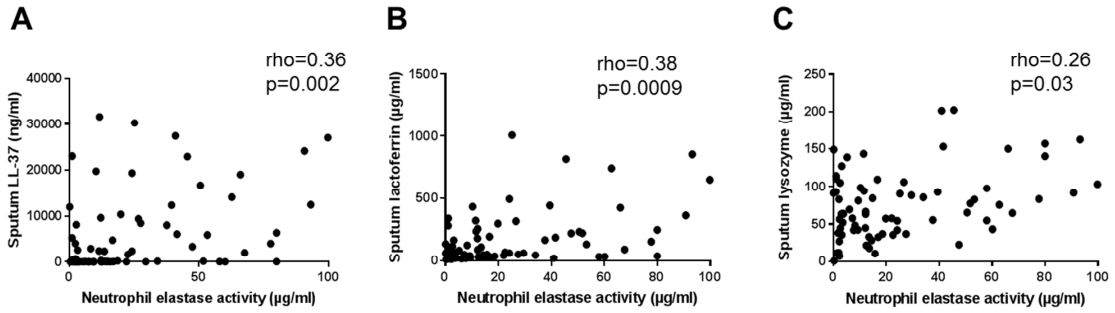
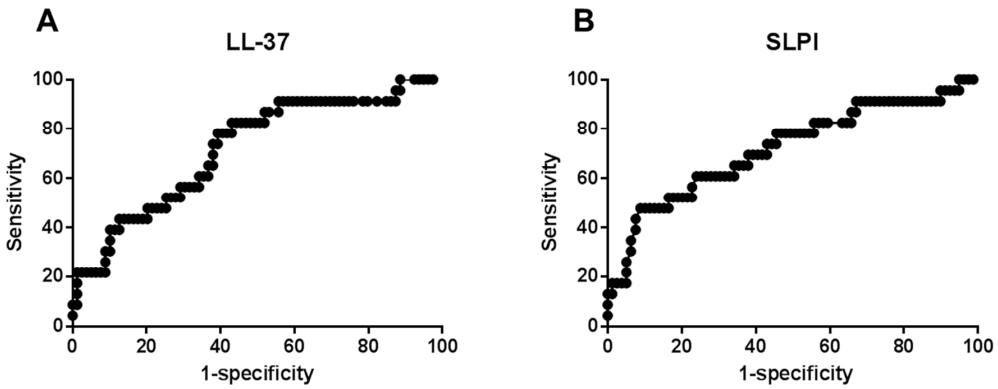

A
rho=0.36
p=0.002
Sputum LL-37 (ng/ml)
Neutrophil elastase activity (µg/ml)
B
rho=0.38
p=0.0009
Sputum lactoferrin (µg/ml)
Neutrophil elastase activity (µg/ml)
C
rho=0.26
p=0.03
Sputum lysozyme (µg/ml)
Neutrophil elastase activity (µg/ml)

Figure E5:


A
LL-37
Sensitivity
1-specificity
0
20
40
60
80
100
B
SLPI
Sensitivity
1-specificity