Antimicrobial peptides in inflammatory phenotypes of COPD

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Introduction

Antimicrobial peptides (AMPs) are released from cells as a line of defence to limit microbial action and colonisation of the epithelial layer. Patients with COPD and neutrophilic (non-eosinophilic) inflammation experience bacterial colonisation frequently (1). The bacteria most commonly found persistently is non-typeable *Haemophilus* influenzae (NTHi) (2). How AMP levels differ amongst COPD inflammatory phenotypes is unknown.

Hypotheses

1. Patients with non-eosinophilic inflammation have lower AMP levels than other COPD inflammatory phenotypes.
2. Presence of NTHi is associated with low AMP levels.

Methods

- Plasma and PBS sputum supernatants from 8 healthy donors, 10 non-eosinophilic asthmatics and 18 COPD patients (Table 1) were tested for SLPI, osteopontin, lysozyme, elafin and beta defensin-1 by ELISA in duplicate.
- Measures below lower limit of detection were excluded from analysis.
- DNA was extracted from sputum plugs with E.Z.N.A. tissue DNA kit. NTHi was measured in sputum plugs by qPCR of the OMP P6 gene, and levels of NTHi quantified against standard plasmid concentrations.
- Patients were stratified into eosinophilic and non-eosinophilic airway inflammation by a 3% sputum eosinophil cut-off.
- Statistical analysis was completed with GraphPad Prism 7.03.

Table 1: Patient characteristics. / = Mean (range), *= Mean (SD) , #= Median (IQR)

	Healthy donors	Asthma	COPD
n	8	10	18
Age (years)/	40 (23-66)	60 (44-68)	68 (45-82)
Gender (M/F)	2/6	4/6	12/6
FEV1 (L) #	2.9 (2.8-3.4)	3.1 (2.6-3.5)	1.4 (1.1-2.3)
FEV1% Predicted*	102.2 (9.4)	84.5 (26.0)	55.7 (20.7)
Smoker (Current/Ex/Non)	0/1/5	0/4/6	6/11/0
Sputum neutrophils %#	41.7 (21.9-66.1)	69.8 (59.7-86.3)	86.1 (83.7-90.9)
Sputum eosinophils %#	0.2 (0.2-2.5)	1 (0.2-5.3)	0.9 (0.2-2.3)
Sputum chocolate agar CFU ($x10^6$) #	0.4 (0.3-0.6)	2.9 (1.0-37.5)	0.4 (0.3-0.7)
NTHi (log gene copies/g)	5.7 (4.8-6.8)	6.0 (4.2-9.1)	5.4 (2.9-6.0)

Results

- Lower limits of detection of AMPs were: SLPI (0.0625ng/ml), lysozyme (0.15ng/ml), osteopontin (0.011ng/ml), elafin (0.0313ng/ml) and beta defensin-1 (0.004ng/ml).
- Between disease groups, median (IQR) beta defensin-1 levels are higher in plasma of COPD patients (10.9ng/ml, IQR 4.2 – 18.1) than healthy individuals (3.7ng/ml, IQR: 2.6 – 4.5, p<0.01) and non-eosinophilic asthmatics (5.0 ng/ml, IQR 3.3 – 7.2, p=0.04), (Figure 1).
- Levels of antimicrobial peptides in plasma and sputum showed no difference between those with non-eosinophilic and eosinophilic COPD (Figure 2). Patients positive for NTHi in sputum (> 10^6 gene copies/ml) were non-eosinophilic.
- No antimicrobial peptide correlated with NTHi levels in the sputum plug (Figure 3).

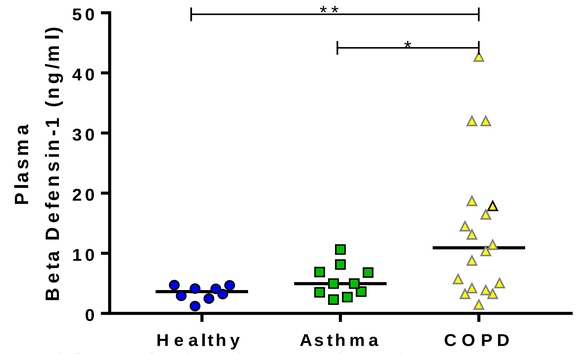

Figure 1: Beta defensin-1 levels in plasma are elevated in COPD patients compared to healthy donors and asthmatics

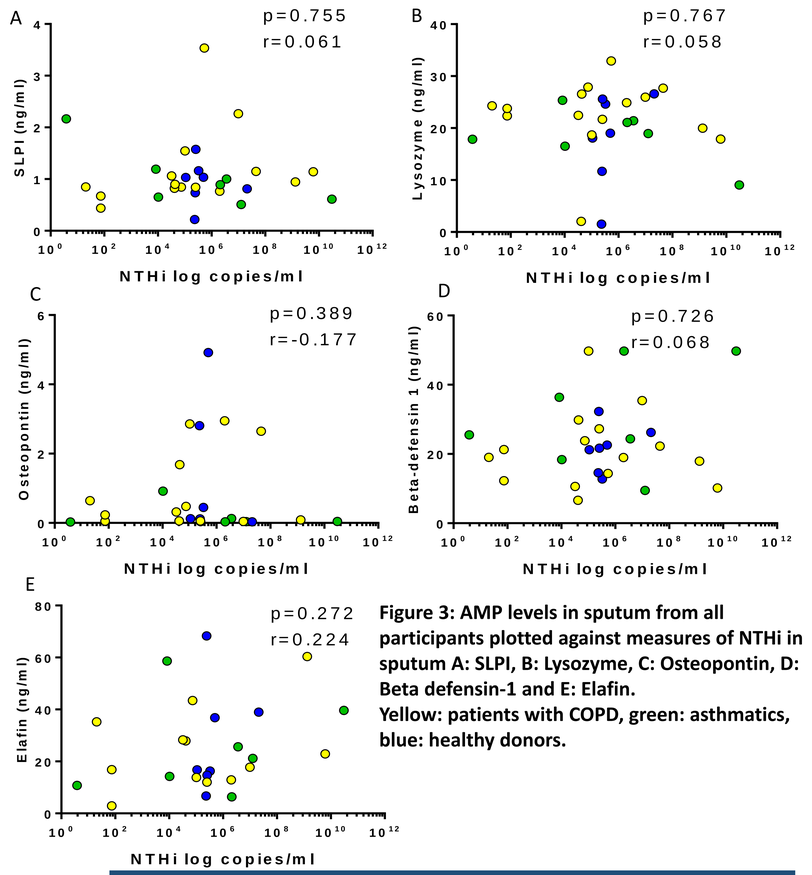

Figure 3: AMP levels in sputum from all participants plotted against measures of NTHi in sputum A: SLPI, B: Lysozyme, C: Osteopontin, D: Beta defensin-1 and E: Elafin.
Yellow: patients with COPD, green: asthmatics, blue: healthy donors.

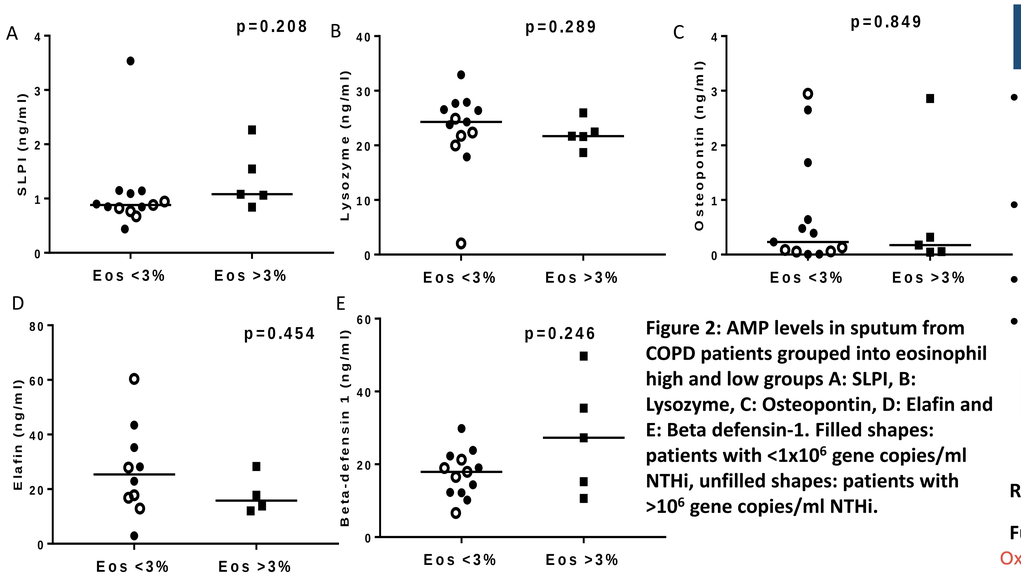

Figure 2: AMP levels in sputum from COPD patients grouped into eosinophil high and low groups A: SLPI, B: Lysozyme, C: Osteopontin, D: Elafin and E: Beta defensin-1. Filled shapes: patients with <1x10^6 gene copies/ml NTHi, unfilled shapes: patients with >10^6 gene copies/ml NTHi.

Conclusion

- Levels of SLPI, osteopontin, lysozyme, elafin and beta defensin-1 are similar between COPD phenotypes in sputum and blood
- This suggests defence against pathogens by these AMPs is not lacking in differential inflammatory COPD phenotypes
- No AMP investigated correlated with NTHi levels in sputum
- The role antimicrobial peptides play in NTHi colonisation remains to be determined.

References

1. Bafadhel et al., *Int J Chron Obstruct Pulmon Dis*., **10**, 1075-1083 (2015)
2. Mallia et al., *Int J Chron Obstruct Pulmon Dis*., **9**, 1119-1132 (2014)

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