Methods Human neutrophils were purified from healthy volunteers by discontinuous percoll gradients. PAO-1 and PHD-deficient strains were grown in lysogeny broth and equivalent growth curves confirmed. Supernatants from wild-type and PHD-deficient *P. aeruginosa* were harvested at two hours and then co-cultured with neutrophils in normoxia and hypoxia. Neutrophil viability and apoptosis was then assessed using AnnexinV/To-pro-3 staining on flow cytometry at three and five hours.

Results Control neutrophils in normoxia saw a decrease in viability of 6% between three and five hours. Neutrophils treated with supernatant from the PHD-deficient strain experienced a decrease in viability from 3139 (± 968) cells at three hours to 2058 (± 586) at five hours – a decline of 34% ($P < 0.05$). Normoxic neutrophils treated with the wild-type strain, however, saw a decrease of 21% ($P < 0.05$).

Hypoxic conditions reversed the killing effects of wild-type *P. aeruginosa:* after five hours neutrophils in normoxia experienced a 21% decrease in viability, whereas the viability of hypoxic cells only decreased by 8% ($P < 0.05$).

Discussion These data highlight the relationship between tissue oxygen tensions and host immunity and that bacteria have evolved virulence factors with novel mechanisms of action; namely preventing neutrophil survival at sites of inflammation. Moreover, the potential oxygen-sensing capabilities of prokaryotes are intricately linked to bacterial virulence.

S45 EVALUATING THE SENSITIVITY AND SPECIFICITY OF ACTIVE NEUTROPHIL ELASTASE AS A BIOMARKER FOR BACTERIAL INFECTION IN SUBJECTS WITH COPD

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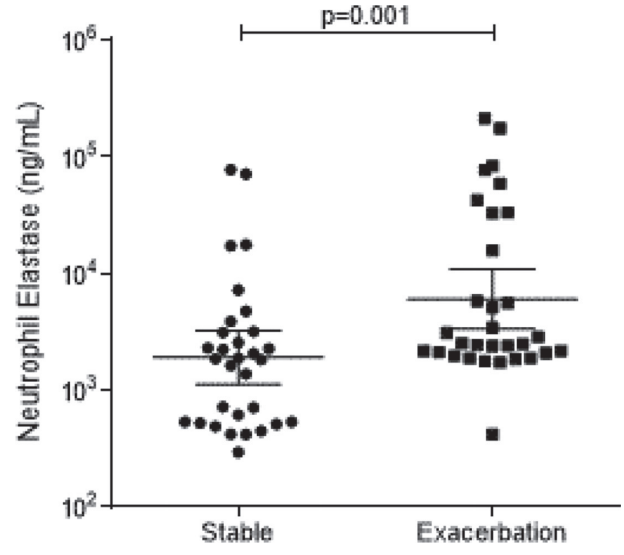
Introduction COPD is a neutrophilic disease, with the majority of subjects having a sputum neutrophil percentage of >60%. Neutrophil elastase (NE) is a serine proteinase, secreted by neutrophils and macrophages during inflammation and has a role in the destruction of bacteria within the host. New advancements now allow accurate assessment of active protease levels in complex biological samples. We sought to investigate if active NE could be used as a biomarker for bacterial infection in subjects with COPD.

Methods NE was quantified using ProteaseTag™ active NE Immunoassay (ProAxsis, Belfast) from cell-free sputum supernatant from 31 COPD subjects (20 Males; mean age 65, range 45 to 81) at stable state and during an exacerbation. Bacterial infection was defined as $\geq 10^7$ CFU/mL in sputum. Subject demographics, sputum cell differential counts and polymerase chain reaction (PCR) for respiratory pathogens were measured.

Results Active NE was higher during an exacerbation compared to stable state (fold difference (95% CI) 0.50 (0.22 to 0.78), $p = 0.001$) (Figure 1). NE correlated with total sputum neutrophils ($p < 0.0001$, $r = 0.48$) and total bacterial load measured by CFU/mL ($p < 0.01$, $r = 0.39$) and qPCR ($p < 0.05$, $r = 0.33$). When looking at the main respiratory pathogens no correlations were seen between *H. influenzae* ($p = 0.43$, $r = -0.11$), S. aureus ($p = 0.34$, $r = -0.14$) or S. pneumonia ($p = 0.11$, $r = 0.23$); however a correlation was seen between NE and *M. catarrhalis* ($p = 0.01$, $r = 0.36$). NE has an area under the receiver operator curve of 0.72 [0.58 to 0.85] to identify a bacterial infection with a sensitivity and specificity of 67.74% and 67.86% at a NE cut off of 2335ng/mL.

Conclusion Active NE is elevated during a COPD exacerbation compared to baseline. Active NE is associated with neutrophilic inflammation and bacteria; and may be a viable biomarker for bacterial infection in COPD.



Abstract S45 Figure 1 Sputum active NE levels at stable and exacerbation state from 31 paired COPD subjects. Mean and 95% CI

S46 NEUTROPHIL VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AS A DRIVING FORCE FOR ANGIOGENESIS IN BRONCHIECTASIS?

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Introduction Bronchiectasis (BR) in a pulmonary disease thought to involve a characteristic dilation of the bronchi resulting from a cycle of airway infection and inflammation. This inflammation is believed to be driven by neutrophils, which are present in the BR lung in high number. Vascular endothelial growth factor (VEGF) is a pro-angiogenic cytokine that may be upregulated in BR and could contribute towards creating a pro-angiogenic airway environment by supporting neutrophil migration into the airway tissue, however this has yet to be shown.

Aims 1) Examine the BR airway for any indications of increased angiogenesis, 2) Assess the ability of neutrophils to secrete VEGF upon stimulation *in vitro*, 3) Evaluate sera/sputa samples VEGF concentration to determine if VEGF could act as a biomarker for BR severity.

Methods Healthy volunteer (HV) and BR endobronchial biopsies were stained with a HRP conjugated anti-CD31 antibody, allowing blood vessels to be counted in a blinded manner. Peripheral blood neutrophils isolated from HV were stimulated (e.g. with TNF-α or bacterial PAMPs) for 4 hours, VEGF levels in supernatants were then quantified using ELISA. A VEGF ELISA was also used to determine VEGF concentration in sera and sputa samples

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