

Abstract S83 Figure 1 Changes in five point asthma control questionnaire (ACQ5), prednisolone use, biomarkers and sputum visual analogue score over time on mepolizumab therapy (n=50 (Month 0), n=46 (M1), n=15 (M4), n=1 (M12)). Bonferroni-corrected paired t tests *, P<0.05, **P<0.01, ****P<0.0001

steroid use and improvements in symptoms, and trends in improved quality of life including reduced anxiety and depression. This confirms the positive impact that Mepolizumab can have on the lives of our patients.

The rise of the foot soldier: neutrophilic inflammation in lung disease

S84 REGULATION OF INTERCELLULAR ADHESION MOLECULE-1 IN HUMAN NEUTROPHILS

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Introduction Neutrophils play a key role in the development of several lung diseases such as COPD and bronchiectasis. Uncovering the mechanisms of essential neutrophil functions may provide novel therapeutic targets for the treatment of these diseases. Intercellular adhesion molecule-1 (ICAM-1) is a glycoprotein shown to support neutrophil phagocytosis and reactive oxygen species production in mouse models. Our study attempts to uncover the role of ICAM-1 in human neutrophils. **Methods** Peripheral blood-derived neutrophils were isolated from healthy donors by plasma-percoll gradients and ICAM-1 expression was determined by flow cytometry using median fluorescence intensity (MFI). ICAM-1 expression was also measured in neutrophils obtained from the pleural fluid compartment of parapneumonic effusion and empyema patients. To measure neutrophil phagocytosis, uptake of pHrodo labelled *S. aureus* was quantified by phagocytic index (PI) in healthy volunteers using a whole blood assay on an Attune NxT Acoustic Focusing Cytometer. An unpaired two-tailed Welch's t-test was used to compare conditions.

Results ICAM-1 expression at 6 hours was significantly increased in neutrophils incubated with 10 ng/mL

lipopolysaccharide (MFI 2814±200 vs 1330±83 (control ±SEM); p<0.001; n=8). Neutrophil ICAM-1 expression also increased following incubation with 30 µg/mL lipoteichoic acid (MFI 2084±110 vs 1284±69 (control); p<0.01; n=4) and 10 ng/mL TNF-alpha (MFI 2333±181 vs 1310±104 (control); p<0.01; n=5). Furthermore, treating neutrophils with the translational inhibitor cycloheximide (n=3) prevented LPS-induced ICAM-1 upregulation. Neutrophils obtained from the pleural fluid of empyema patients (n=5) show dramatically increased levels of ICAM-1 expression compared to parapneumonic effusion (n=3) neutrophils (MFI 1526±207 vs 6373±769 (empyema); p<0.01). Treating blood neutrophils with cell-free empyema pleural fluid is also sufficient to induce ICAM-1 expression (MFI 2925±672 vs 1447±147 (control); n=2). Using the whole blood assay, we show that neutrophils expressing high levels of ICAM-1 (75th percentile) have a higher phagocytic index compared to ICAM-1 low (25th percentile) populations (PI 1057100±89 212 vs 817573±1 03 106 (low); n=5).

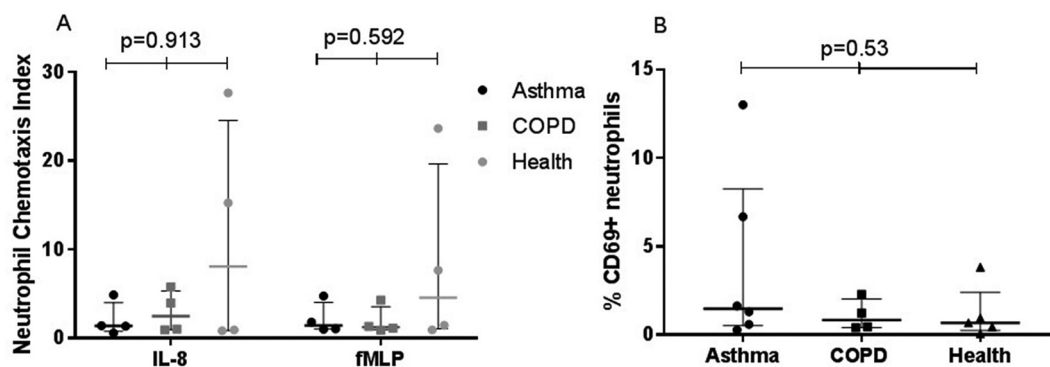
Discussion Overall, our data provide insights into the regulation of ICAM-1 expression in blood-derived neutrophils and support a potential role for ICAM-1 in neutrophil phagocytosis. Further work is underway investigating the mechanisms underlying ICAM-1 expression in pleural fluid neutrophils, and the relationship between ICAM-1 expression and phagocytic capacity.

S85 NEUTROPHIL STATUS AND CHEMOTAXIS IN AIRWAYS DISEASE

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Introduction Neutrophil levels are commonly raised in circulation in COPD and to a lesser extent in asthma, and may play a role in the pathophysiology of these diseases. We hypothesised that the mobility and status of neutrophils is altered in



Abstract S85 Figure 1 A) median (IQR) of neutrophil chemotaxis index. B) median (IQR) percentage of blood neutrophils that express CD69 in patient groups

disease. Neutrophil ability to migrate in response to chemoattractants interleukin (IL)-8 and fMLP was investigated, and their expression of activation markers CD11b, CD69 and CD62L was assessed.

Methods Whole blood was collected from 4 asthmatics, 4 patients with COPD and 4 healthy donors. Granulocytes were isolated using ficoll-hypaque and dextran separation. Chemotaxis was assessed towards 10 nM fMLP and 20 nM through a 5 μ m pore ChemoTx. After 1 hour, Cell-Titre-Glo solution was added to the cells that had moved through the membrane, with luminescence measured by plate reader. Chemotactic index was calculated by dividing the number of migrated cells in response to chemoattractant by the number of cells migrated to media alone. Whole blood was stained for flow cytometry with CD16, CD11b, CD69 and CD62L antibodies. Neutrophils were gated as FSC^{hi}SSC^{hi}CD16⁺ cells. Multiple groups were compared by Kruskal-Wallis test.

Results A non-significant trend to a higher degree of chemotaxis in the healthy controls compared to asthmatics and COPD was seen (see figure 1A). No differences in activation markers between disease groups were seen (figure 1B).

Conclusion Neutrophils from both asthma and COPD patients show a trend to less migration towards their chemoattractants IL-8 and fMLP than neutrophils from healthy donors. This decreased mobility may contribute to the increased incidence of infections due to impaired bacterial clearance.

S86

ROLE OF OXIDATIVE STRESS IN SEVERE ASTHMA

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Introduction Asthma affects 300 million people world-wide and is severe in 10% of sufferers. Existing literature suggests that oxidative stress is implicated in asthma pathogenesis, however, there is limited data characterising its role in severe asthma. It's not fully established whether higher levels of oxidative stress are associated with specific aetiological factors, such as bacterial infection and airway inflammation.

Aims I sought to quantify sputum differential cell counts, bacterial load and oxidative stress levels in severe asthmatics, assess relationships between these parameters and ascertain whether they impact on asthma control, and risk of exacerbations.

Methods An 8-oxodG ELISA quantifying oxidative stress and quantitative PCR measuring total bacterial load were performed on sputum supernatant of 128 subjects, attending a tertiary referral severe asthma centre. Sputum differential cell counts were measured and subjects were stratified according to severity of oxidative stress (high >2 ng/ml 8-oxodG) and bacterial load (high >10⁷ copies/ml total 16S). Longitudinal asthma control (ACQ6) and exacerbations were recorded.

Results Higher sputum neutrophils were observed in oxidative stress^{HIGH} subjects versus oxidative stress^{LOW} (Mann-Whitney median (IQR) 81.5 (59.25-95) vs 64.6 (38.9-85.31), $p=0.020$). Sputum neutrophil percentage and bacterial load showed positive correlation ($r=0.29$, $p=0.0009$) however, eosinophils were negatively correlated with total 16S levels ($r=-0.23$, $p=0.0086$). 4 groups formulated upon bacterial load and oxidative stress levels, showed a significant difference in neutrophil count (Kruskal-Wallis $p=0.045$); oxidative stress^{HIGH}/bacterial load^{HIGH} subjects had the highest neutrophil count versus subjects that were oxidative stress^{LOW}/bacterial load^{LOW} (Mann-Whitney median (IQR) 81.66 (72.5-95) vs 64.6 (38.9-85.94), $p=0.0074$). There was no difference in baseline nor change in ACQ between these groups existed, but those that were oxidative stress^{LOW}/bacterial load^{LOW} had a lower exacerbation frequency (Kruskal-Wallis $p=0.027$) (table 1).

Abstract S86 Table 1 Median exacerbations/year in groups based on oxidative stress levels and bacterial load

	Total 16s high and 8oxodG high	Total 16s high and 8oxodG low	Total 16s low and 8oxodG high	Total 16s low and 8oxodG low
Median (IQR) exacerbations/year	2.61 (1.00-4.00)	2.49 (1.32-4.07)	3.78 (2.70-6.33)	1.10 (0.08-2.71)

Discussion Oxidative stress together with bacterial load are associated with neutrophilic inflammation. Future exacerbation risk was lowest in asthmatics with a low bacterial load burden and oxidative stress. Whether oxidative stress is a consequence or cause of bacterial load and neutrophilic inflammation remains unknown, but my findings suggest that studying treatments targeting oxidative stress in asthma and the consequent effects upon neutrophilic inflammation, bacterial load and clinical outcomes is warranted.