Results 33 patients grew at least one new isolate of NTM: MABSC (n = 20, 5 of which grew other NTM species in temporally distinct episodes), MAC (n = 12) & other NTMs (n = 5, mainly *M. kansasii*). 51% female. Median age at 1st isolation 12.3 yrs (range, 4–17.3), and FEV1 79.5% predicted (50–116%). 10 (26%) initial isolates were from BAL. For MABSC: 4 (20%) had ABPA and 6 (30%) CFRD. All patients met ATS/IDSA criteria for diagnosis and were treated in accordance with national consensus guidelines. Spontaneous clearance was seen in 100% of other NTM infections. For data on clearance and treatment of MABSC and MAC, see Table 1. 9/14 who completed NTM treatment showed culture conversion at 3 months. Only 2 children with negative cultures at 3 months went on to have subsequent positive microbiology.

Conclusion This is the first report discussing treatment success for NTM in a large paediatric cohort. Although single centre, there is a similar incidence of NTM to that reported for adult CF populations. Spontaneous clearance is more common with MAC (42%) and other NTM infections compared to MABSC (10%). To date 53% of treated MABSC are considered eradicated 12 months post treatment. Early culture conversion appears to be linked with treatment success. Further studies are needed to identify if a lack of early clearance should identify children appropriate for further inpatient induction therapy.

Abstract S42 Table 1 Treatment and eradication of *M. abscessus* complex (MABSC) and *M. avium* complex (MAC)

	MABSC	MAC
n	20	12
Spontaneous clearance, n (%)	2 (10%)	5 (42%)
Commenced treatment, n (%)	17 (85%)	5 (42%)
Days to treatment from 1st isolation, median (range)	113 (15– 587)	518 (42– 698)
Treatment duration in months, mean (range)	24.6 (15– 48)	18.5 (14– 22)
Completed treatment, n (%)	10 (59%)	4 (80%)
Eradication with treatment, n (%)	9 (53%)	3 (60%)
Relapsed after treatment, n (%)	2 (12%)	0

Innate Immunity in Lung Disease

S43 HYPOXIA UPREGULATES PI3KINASE-DEPENDENT NEUTROPHIL DEGRANULATION AND NEUTROPHIL-MEDIATED TISSUE INJURY

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10.1136/thoraxjnl-2016-209333.49

Introduction Damage to host tissue from persistent neutrophilic inflammation is implicated in the pathogenesis of many diseases, including chronic obstructive pulmonary disease (COPD). Infected/inflamed tissues can be profoundly hypoxic; this state may synergise with inflammatory cytokines to promote a destructive neutrophil phenotype with enhanced potential for tissue damage.

Methods Neutrophils isolated from COPD patients or healthy volunteers were incubated under normoxia (21% O2) or hypoxia (0.8% O2) before treatment with priming (GM-CSF/PAF/TNF-α) and stimulating (fMLP) agents, with/without PI3Kinase inhibitors (pan/γ/δ). Neutrophil elastase (NE) activity was measured by Enzchek® assay. Western blotting for total and phosphorylated Akt was performed using cell lysates. Neutrophil extracellular trap (NET) production was assessed by fluorescence absorbance. Neutrophil supernatants were incubated with primary human pulmonary artery endothelial cells (HPAEC); death and detachment were measured by MTT assay and confocal microscopy. Precipitated neutrophil supernatants were separated by SDS polyacrylamide gel electrophoresis (PAGE) and silver stained. S100A8/A9 homo- and heterodimer content of neutrophil supernatants was assessed by ELISA.

Results Hypoxia increased NE release in an agonist- and PI3K-γ-dependent manner, with more pronounced hypoxic degranulation responses seen in exacerbating COPD patients. Hypoxia augmented resting and cytokine-stimulated Akt phosphorylation; PI3K-γ inhibition abrogated Akt phosphorylation and prevented the hypoxic uplift of NE release. Hypoxia did not increase NET production in resting or GM-CSF/fMLP treated cells. Hypoxic neutrophil supernatants induced extensive HPAEC detachment and death, which was prevented by co-incubation with alpha-1 antitrypsin. Silver stained protein bands from precipitated neutrophil supernatants separated by SDS-PAGE were identified by mass spectrometry, suggesting a hypoxic increase in damage associated molecular pattern (DAMP) proteins S100A8 and S100A9. When interrogated by ELISA, there was no difference between the amount of S100A8/A9 hetero- or homodimers in normoxic versus hypoxic supernatants.

Conclusion Hypoxia augments neutrophil degranulation in an agonist- and PI3K-γ-dependent manner, which may be further increased during COPD exacerbations. Hypoxic neutrophil supernatants have enhanced capacity to damage endothelial cells *in vitro*, likely due to increased release of NE. The contribution of S100A8/A9 proteins to this damage is currently unclear. Hence, hypoxia promotes a destructive histotoxic neutrophil phenotype with potential relevance to diseases such as COPD.

S44 PSEUDOMONAS AERUGINOSA INDUCES NEUTROPHIL CELL DEATH WHICH IS REVERSED BY HYPOXIA

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10.1136/thoraxjnl-2016-209333.50

Introduction Neutrophils accumulate in the lungs of patients with bronchiectasis and cystic fibrosis, and the resulting inflammation causes tissue hypoxia. Neutrophils have a unique ability to survive and function in such hypoxic environments, a response regulated by the hypoxia-inducible transcription factor (HIF)/hydroxylase oxygen sensing pathway.

Pseudomonas aeruginosa is an opportunistic pathogen which colonises patients with chronic lung disease, including cystic fibrosis. It secretes the toxin pyocyanin, which induces neutrophil apoptosis in an oxygen-dependent manner, as a means of immune-evasion. *P. aeruginosa* has recently been shown to possess hydroxylase-homologs, suggesting that prokaryotes may also have oxygen-sensing capabilities. A prolyl-hydroxylase (PHD)-deficient strain secretes higher levels of pyocyanin compared with wild-types.

We hypothesised that PHD-deficient *P. aeruginosa* induces a higher degree of neutrophil death compared with an otherwise genetically identical wild-type strain. Furthermore, we postulated that the killing effects of these bacteria are reversed in hypoxia.