T3 SEVERE HYPOXIA EXISTS WITHIN PULMONARY TUBERCULOSIS LESIONS AND AUGMENTS MATRIX METALLOPROTEINASE-MEDIATED IMMUNOPATHOLOGY

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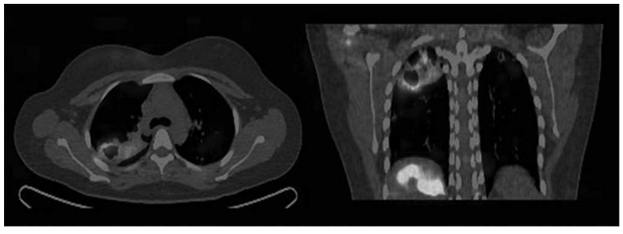
Introduction Mycobacterium tuberculosis (MTb) causes approximately two million deaths each year. Extensive lung destruction is a hallmark of pulmonary tuberculosis and is caused by the breakdown of lung extracellular matrix by host matrix metalloproteinases (MMPs). Hypoxia upregulates gene expression and secretion of many inflammatory mediators via hypoxia-inducible factor (HIF-1). Hypoxia has been demonstrated in several animal models of TB infection but no studies have been performed to investigate hypoxia in humans.
Methods To investigate the presence of hypoxia in human *M.tb* infection we performed PET-CT scanning using the hypoxia tracer [18F]FMISO in patients with pulmonary infection. Next, primary human monocyte-derived macrophages (MDMs) and human respiratory epithelial cells were stimulated by *M.tb* H37RV or conditioned media from Mtb-infected monocytes (CoMTb) in a specially commissioned hypoxia workstation at 1% O2, 5% CO2. MMPs and their specific inhibitors (TIMP-1/2) were analysed by ELISA, Luminex array, zymography and confocal microscopy. Gene expression was analysed by qPCR and promoter activity by dual luciferase activity. Total HIF-1 was measured by western analysis. The effect of HIF-1 blockade was investigated using HIF-1a siRNA.
Results PET-CT scans demonstrated evidence of tracer trapping in regions of consolidation and in regions surrounding pulmonary cavities indicating severe hypoxia. Hypoxia potently upregulated MMP-1 in *M.tb*-stimulated primary MDMs and MMP-1 and MMP-9 in respiratory epithelial cells compared to normoxia ($p < 0.001$). Similarly, hypoxia increased MMP-1 gene expression -2500 fold and potently increased MMP-1 promoter activity ($p < 0.001$). Site-directed mutagenesis of the NF B binding site in the MMP-1 promoter and SC514 and helenalin chemical inhibition demonstrated that increased MMP-1 activity in hypoxia was regulated via an NF B dependent mechanism. *M.tb* infection caused stabilisation of HIF-1 protein even under conditions of normoxia but was more pronounced in hypoxia. Immunohistochemistry of patient samples demonstrated strong HIF-1 immunoreactivity in tuberculosis patients compared to controls. HIF-1 siRNA confirmed that increased MMP-1 activity in hypoxia occurred by a HIF-1 -dependent mechanism.



Abstract T3 Figure 1. Axial and coronal PET-CT images of a female patient with pulmonary Tb. Trapping of the hypoxia tracer 18F-FMISO in TB-infected regions confirms severe hypoxia.

Conclusion We demonstrate for the first time that severe hypoxia develops within pulmonary TB lesions. Hypoxia increases *M.tb*-driven MMP-1/9 gene expression and secretion in a HIF-1a-dependent manner. These results support the hypothesis that during Tb infection, hypoxia develops and promotes immunopathology.

T4 A CROSS-SECTIONAL ANALYSIS OF THE EFFECT OF COPD ON WORK CAPABILITY USING THE BIRMINGHAM COPD COHORT

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Introduction The effect of COPD on work is poorly understood. Approximately 40% are of working age, however, employment rates are lower compared to others. Little is known about characteristics of COPD patients who remain in employment compared to those who do not.
Aim To assess which factors are associated with employment status among working age COPD patients.
Methods 2000 patients with COPD from primary care are being recruited into a three-year cohort study. In addition to clinical data, occupational history and work performance are assessed. Interim baseline data was used to assess associations between employment status, co-morbidities, COPD assessment test (CAT) scores (impact of COPD on HRQL), lung health, BMI and exercise capacity among COPD patients of working age.
Results Of the 1094 patients recruited 31.8% (n = 348) were of working age (mean age 54.9), of whom 31.6% (n = 110) were in work. Compared to those not in work, working patients were more likely to be male (72.7% vs 52.5%) but were similar in terms of smoking history (ever smokers: 94.4% vs 91.3%).

Overall, working patients had fewer co-morbidities. Adjusted for age, sex and smoking status, they were less likely to have cardiovascular disease (OR = 0.58; 95% CI 0.38–0.87), gastrointestinal disease (OR = 0.37; 0.20–0.69), cancer (OR = 0.09; 0.01–0.72)) and osteoarthritis (OR = 0.4; 0.19–0.84). Being in work was associated with less dyspnoea (OR = 0.12; 0.05–0.25 for MRC grade 1 vs. grade 5), higher quality of life (OR = 0.34; 0.14–0.85 for low vs. very high CAT score) and a greater exercise capacity (OR = 1.14; 1.08–1.19), but BMI was not associated with employment status (OR = 1.0; 0.97–1.06). There was no difference in disease severity (assessed by GOLD stage) between the two groups (p for trend = 0.16).
Conclusions This is the first primary care study in the UK to consider the impact of COPD on work capability. COPD patients who are in work tend to be healthier, are less breathless and have a better quality of life than those not in work, even though disease severity in the two groups was similar. The findings from this analysis suggest that being in work is good for health but longitudinal studies are needed to confirm this.

T5 CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN SMOKERS AND PATIENTS WITH COPD ARE DYSFUNCTIONAL DUE TO INCREASED DNA DAMAGE AND SENESCENCE

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Introduction Cardiovascular disease (CVD) is a major cause of death in smokers, particularly in patients with chronic obstructive pulmonary disease (COPD). Circulating endothelial progenitor cells (EPC) are required for endothelial homeostasis, and their dysfunction contributes to CVD. DNA damage has been recognised as an important contributor to CVD. Our aim was to investigate whether EPC from smokers and COPD patients are dysfunctional, and the role of DNA damage pathways in mediating endothelial dysfunction in these patients.

Methods To investigate EPC dysfunction in smokers, we isolated and expanded blood outgrowth endothelial cells (BOEC) from peripheral blood samples of healthy non-smokers, healthy smokers and COPD patients. Endothelial senescence was measured by senescence-associated -galactosidase (SA- -Gal) activity. Expression of sirtuin (SIRT)-1, p16, p21, -H2AX and 53BP1 were measured by Western blotting and/or immunofluorescence confocal microscopy. SIRT1 activity was measured using a SIRT1 fluorescent activity assay kit. To investigate angiogenesis *in vivo*, BOEC were labelled with Vybrant DiI Cell-Labelling Solution, mixed with Matrigel and injected subcutaneously into the back of NOD. CB17-Prkdcscid/NcrCrl mice. Seven days later, the mice were sacrificed and the plugs were cryosectioned.

Results BOEC from smokers and COPD patients showed increased DNA double-strand breaks (measured by -H2AX, 53BP1) and senescence (senescence associated- -galactosidase activity, p16 and p21 levels) compared to non-smokers. Senescence negatively correlated with sirtuin-1 (SIRT1) expression and activity, a protein deacetylase that inhibits DNA damage and cellular senescence. Inhibition of DNA damage response by silencing of ataxia telangiectasia-mutated (ATM) kinase resulted in up-regulation of SIRT1 expression and decreased senescence. Interestingly, treatment of BOEC from COPD patients with the SIRT1 activator resveratrol or a selective ATM inhibitor rescued the senescent phenotype. Using the *in vivo* Matrigel plug assay, BOEC from COPD patients displayed reduced angiogenesis (capillary-like structures) and increased DNA damage, senescence and apoptosis (measured by 53BP1, p16, TUNEL and cleaved-caspase 3 staining) compared to non-smokers.

Conclusions BOEC from smokers and COPD patients show reduced angiogenesis *in vivo* and display increased DNA damage and senescence, associated with reduced SIRT1 expression. These defects may contribute to endothelial dysfunction and cardiovascular events in smokers and COPD patients and could potentially constitute therapeutic targets for intervention.

T6 THE ROLE OF IL-17A IN A MOUSE MODEL OF PULMONARY INFECTION CAUSED BY STREPTOCOCCUS PNEUMONIAE IS STRAIN DEPENDENT

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Background The cytokines interleukin 17A and interleukin 22 are known to be important in host defence against Gram-negative infection. However, their role in the innate immune response to pneumococci remains poorly understood. We aimed to investigate the role of these cytokines in an animal model of pneumococcal infection.

Methods Wild type (WT), IL-17 receptor A-/- (IL17RAKO) and IL-22-/- C57Bl/6 mice were infected intranasally with serotype 4 (TIGR4) and serotype 3 (SRL1) pneumococci. Neutrophils for *in vitro* experiments were obtained by peritoneal lavage following injection of casein.

Results TIGR4 was cleared from the lungs of mice within 48 hours but invaded early in the course of the infection causing bacteraemia and disseminated infection. In contrast, SRL1 invaded into blood late in the course of infection but caused a dense consolidative pneumonia with purulent empyema. IL-17A and IL-22 were detected in alveolar lavage within 6 hours of infection but fell in later infection suggesting an innate source. Contrasting results were obtained when knockout animals were infected. In TIGR4 infection, IL17RAKO were more susceptible to infection ($P = 0.04$) and this was due to an increased incidence of early bacteraemia in these animals. In contrast, following SRL1 infection in IL17RAKO animals, there was improved survival ($P = 0.004$). In both types of infection, IL22KO animals demonstrated an intermediate phenotype that did not reach statistical significance. IL17RAKO mice had decreased neutrophils in blood and lung within 24 hours of infection in keeping with the known biological actions of IL-17A. Studies using isolated cells showed that TIGR4 was phagocytosed and killed by neutrophils but uptake and killing of SRL1 was ineffective. Experiments with fluorescent bacteria confirmed poor neutrophil uptake of SRL1 relative to TIGR4. Depletion of WT mouse neutrophils with a monoclonal antibody demonstrated a trend towards delayed mortality and improved outcome in SRL1 infection.