the main contributor to disease activity. The most effective treatment, corticosteroids, has a high adverse effect burden so there is a need to define more specific therapeutic agents with less side effects. In addition, for some patients with progressive active disease, corticosteroids are often unhelpful; therefore better understanding of the mechanisms of disease is needed for development new drugs. This study examines the potential immune processes involved in disease activity in sarcoidosis using gene expression profiling of peripheral blood mononuclear cells (PBMCs) (n=29) and bronchoalveolar lavage cells (BALCs) (n=12). Patients with well-defined pulmonary sarcoidosis and secure tissue-supported diagnosis were recruited from the Oxford Sarcoidosis Service during a defined 2 year period. Patients were not on treatment at the point of sampling. A CTAS¹-validated chest radiograph-blood disease activity score comprising lymphocyte, ACE and IgG levels (SCAS, scores from 0 to 12 reflecting low to high activity) was used to measure activity at the point of sampling. Gene expression profiles were derived from PBMC and BALCs using the Illumina HT-12 v4 expression chip. All RNA had a RIN $\geq$ 8. We found a significant positive correlation between the 'immune response' gene set and SCAS for BALCs, by GSEA and Metacore functional analyses. Within this gene set, a transcriptional signature related to monocyte activity and function was shown to be the most significant gene network with an unexpected downregulation of TGF β receptor signalling pathway in low activity BALCs. In PBMCs and BALCs, the two IFN-g-inducible, monocyte-produced genes CXCL-9 and CXCL-10 were the soluble factors that most correlated with increasing activity ($r\geq 0.5$ by Spearman Correlation). In an independent cohort, SCAS levels were examined against CD14^{hi} classical monocyte levels (n=40, same inclusion criteria). This showed a marked correlation between monocyte frequency and level of activity as measured by SCAS ($r=0.67$; $p<0.001$; Spearman Rank correlation). These Results implicate monocytes as a major contributor to disease activity in sarcoidosis and propose monocyte pathways as potential specific targets for new therapeutics in sarcoidosis.

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S78

INCREASED CD16BRICD62LDIMCD11B+SUBSET OF NEUTROPHILS IN BRONCHOALVEOLAR LAVAGE FROM PATIENTS WITH INTERSTITIAL LUNG DISEASE

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Introduction The Interstitial Lung Diseases (ILD) are a heterogeneous group of inflammatory and fibrotic diseases of the interstitium, with worst cases resulting in pulmonary fibrosis (PF). Increased neutrophils are found within the lung or bronchoalveolar lavage (BAL) in ILD and predict a poor prognosis. Neutrophil adhesion molecules, e.g., CD18/b2 integrins (LFA-1; CD11a, Mac-1; CD11b and CR3; CD11c) and L-Selectin (CD62L) regulate cellular recruitment and fibrosis in animal models of bleomycin-induced PF. Expression of the Fc receptor (CD16) is upregulated during neutrophil activation, whilst ICAM-1 (CD54) is a marker for neutrophil reverse-transmigration back across the endothelium.

Study Aim Investigate adhesion molecule expression profile of neutrophils in ILD patients compared to controls.

Methods BAL samples were collected from ILD and non-ILD patients undergoing bronchoscopy with informed consent. Adhesion molecule expression was studied via flow cytometry by staining cells with CD16, CD62L, CD11b, CD11c, CD11a, CD18 and CD54 antibodies.

Results Flow cytometric analysis of BAL showed significantly more neutrophils in ILD lavage express CD11b and CD18 compared to non-ILD controls ($p=0.0016$ and $p=0.0211$ respectively). No significant differences were found in CD11c or CD11a expression. Further analysis revealed ILD lavage contained a higher percentage of CD16^{bric}CD62L^{dim} neutrophil subset expressing CD11b than non-ILD lavage controls ($p<0.0001$); a subset previously associated with a suppressive phenotype.¹ In addition, ICAM-1 expression was significantly down-regulated in ILD lavage neutrophils ($p=0.0397$) and this was also reflected in the CD16^{bric}CD62L^{dim} neutrophil population ($p=0.0445$).

Conclusions From our preliminary study, we have observed an increased percentage of CD16^{bric}CD62L^{dim}CD11b⁺ subset of neutrophils in ILD lavage compared to controls. ILD lavage neutrophils express significantly less ICAM-1. This suggests that more neutrophils are entering and being retained within the lung in ILD. Further experiments will dissect whether ILD neutrophils have altered functions (such as NETosis, ROS production, adhesion or migration) to contribute to disease progression.

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S79

LOCALISED HYPOXIA ENHANCES NEUTROPHIL EXTRAVASATION AND ACTIVATION IN INTERSTITIAL LUNG DISEASE

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Background Neutrophilic inflammation is common in various diseases and may contribute to the pathophysiology of interstitial lung disease (ILD), however the underlying mechanism are not fully understood. Localised tissue hypoxia is often accompanied with inflammation, which may alter cellular responses to drive immunopathology.

Hypothesis We propose that hypoxia modulates neutrophil functions including integrin activation, neutrophil extravasation and neutrophil extracellular trap (NET) release that may contribute to pulmonary damage in ILD

Methods Pulmonary hypoxia was assessed using both fluoromisonidazole (FMISO)-PET scanning of ILD patients and immunohistochemical HIF-1 α staining in ILD and control lung sections. To examine the effects of hypoxia, isolated neutrophils were cultured under hypoxia (1% oxygen) or normoxia (21% oxygen) prior to experimentation. Neutrophil integrin expression was evaluated using flow cytometry. Neutrophil

adhesion and trans-endothelial migration were measured using fluorescence-based assays. Reactive oxygen species (ROS) production was measured using an enzymatic assay to assess hydrogen peroxide (H_2O_2) generation. NETosis was measured using a novel capture ELISA. Bronchoalveolar lavage (BAL) were obtained from ILD or control patients and assessed for NETs.

Results FMISO-PET scans indicated localised hypoxia in fibrotic regions in ILD patients. Hypoxia was also determined in lung sections, which demonstrated positive HIF-1 α in ILD but not control lung sections. ILD-BAL had significantly greater levels of NETS compared to control ($p=0.0039$). Hypoxia did not affect b_1 integrin expression, however increased surface expression of the α_M and α_X subunits ($p=0.0001$ and 0.0179 respectively). Unstimulated, PMA- and LPS-stimulated neutrophil adhesion to both resting and activated endothelial monolayers were enhanced under hypoxia. Neutrophil trans-endothelial migration, across both resting and activated endothelial cells, was also greater in hypoxia ($p<0.05$). Interestingly, whilst ROS generation was not affected by hypoxia, both spontaneous and PMA-induced NETosis were increased under hypoxic conditions ($p<0.05$ and 0.001 respectively).

Conclusions We have demonstrated that localised hypoxia is a feature of the ILD lung. Moreover, elevated NETs were found in ILD-BAL, suggesting a role for aberrant neutrophil activation in ILD pathology. Hypoxia increased neutrophil b_2 integrin expression, adhesion to endothelial monolayers, trans-endothelial migration and NETosis. Further work is underway to investigate the signalling pathways underlying neutrophil activation in ILD.

Changes in pulmonary rehabilitation: new diseases, new approaches

S80

A FEASIBILITY STUDY OF A MULTI-CENTRE RANDOMISED CONTROLLED TRIAL OF ASTHMA-TAILORED PULMONARY REHABILITATION (AT-PR) VERSUS USUAL CARE (UC) IN INDIVIDUALS WITH SEVERE ASTHMA

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Introduction There is limited evidence regarding the acceptability and efficacy of pulmonary rehabilitation (PR) for patients with severe asthma. We investigated the feasibility of performing a randomised controlled trial of asthma-tailored pulmonary rehabilitation (AT-PR) versus usual care (UC).

Method Patients with severe asthma were recruited and randomised 2:1 to AT-PR or UC. The primary outcome was recruitment, retention and adverse event rates. Secondary outcome measures were assessed before and after a 12 week intervention period and included the incremental shuttle walk test (ISWT), two cardiopulmonary exercise tests on a treadmill (ITM) and cycle-ergometer (ICE), quadriceps muscle strength, the asthma control questionnaire (ACQ), chronic respiratory questionnaire (CRQ), asthma quality of life questionnaire (AQLQ), hospital anxiety and depression scale (HADS) and measures of airway inflammation (sputum eosinophil count

and fractional exhaled nitric oxide [FeNO]). Analysis of covariance adjusting for baseline differences was used to compare the effect of ATPR compared to UC. By recruiting 60 patients, a recruitment rate of 30% was estimated with a 95% CI of $\pm 7\%$.

Results 61 out of 235 eligible patients were recruited (26%); 38 females, mean (SD) age 54 (13) yr, BMI 32 (7) kg/m², FEV₁ 1.95 (0.74) L, FEV₁/FVC 69 (11)% and 51 were randomised to AT-PR ($n=34$) or UC ($n=17$). The retention rates for AT-PR and UC were 62% and 53%, respectively. There were 7 serious adverse events but none related to the AT-PR sessions. The Results for the secondary outcome measures are shown in Table 1.

Conclusion AT-PR may be effective for patients with severe asthma but any future trial design would need to be modified to improve retention rate. In this small study, changes in airway inflammation were similar between AT-PR and usual care.

Abstract S80 Table 1 Comparison of exercise performance, asthma control, health related quality of life, depression and anxiety and airway inflammation between AT-PR and UC.

	AT-PR Group (n=21)		UC Group (n=9)		Difference between AT-PR and UC
	Before	After	Before	After	
ISWT	418	450	443	403	74
(m)	(172)	(199)	(121)	(104)	(25 to 124)
ITM VO_{2pk} (ml/kg/ min)	22.5	23.2	22.9	22.3	1.4
	(6.2)	(7.6)	(3.9)	(2.4)	(-1.1 to 3.8)
ICE VO_{2pk} (ml/kg/ kg)	17.5	18.8	16.9	16.1	2.0
	(6.0)	(6.9)	(4.3)	(2.8)	(-0.3 to 4.2)
QMVC	103	116	113	110	22
(Newton)	(53)	(64)	(35)	(28)	(4 to 41)
ACQ	2.1	1.9	1.5	1.9	-0.4
	(0.8)	(0.8)	(0.9)	(1.0)	(-0.9 to 0.1)
CRQ-D	3.1	4.1	3.7	3.6	0.9
	(1.2)	(1.3)	(1.5)	(1.1)	(0.1 to 1.8)
CRQ-F	3.7	4.7	4.7	4.4	0.9
	(1.1)	(1.2)	(1.7)	(1.6)	(-0.1 to 1.8)
CRQ-EF*	5.1	5.4	6.1	6.1	-0.1
	(1.4)	(1.1)	(0.7)	(0.8)	(-0.6 to 0.5)
CRQ-M*	4.7	5.4	6.1	6.0	0.2
	(1.3)	(1.1)	(1.0)	(0.8)	(-0.5 to 0.9)
AQLQ*	19.0	19.7	22.2	21.7	-0.2
	(4.5)	(4.0)	(3.0)	(2.6)	(-2.7 to 2.3)
HADS-A*	6.3	5.1	3.2	2.4	0.4
	(4.4)	(3.7)	(2.5)	(2.8)	(-1.3 to 2.1)
HADS-D*	4.8	3.5	2.8	2.4	-0.3
	(2.7)	(2.8)	(1.6)	(1.0)	(-1.9 to 1.3)
Sputum	-0.1	0.1	0.6	-0.4	0.3
Eos #	(1.0)	(0.8)	(1.0)	(0.6)	(-0.6 to 1.2)
FeNO	33	30	36	37	-4
(ppb)	(23)	(22)	(16)	(16)	(-14 to 5)

Data presented as mean (SD) for before and after intervention results and mean (95% CI) for changes between group.

*significant difference at baseline between groups, # data presented as geometric mean (log SD).

AT-PR; asthma-tailored pulmonary rehabilitation, UC; usual care, ISWT; incremental shuttle walk test, ITM; maximal incremental treadmill test, VO_{2pk}; peak oxygen uptake, ICE; maximal incremental cycle-ergometer test, QMVC; quadriceps maximal voluntary contraction, ACQ; asthma control questionnaire, CRQ; chronic respiratory questionnaire, CRQ-D; CRQ Dyspnoea, CRQ-F; CRQ Fatigue, CRQ-EF; CRQ Emotional Function, CRQ-M; CRQ Mastery, AQLQ; Asthma Quality of Life Questionnaire, HADS; Hospital Anxiety and Depression Score, HADS-A; HADS Anxiety, HADS-D; HADS Depression, EOS; eosinophil count, FeNO; fractional exhaled nitric oxide, ppb; parts per billion.