significant reductions ($p < 0.01$) in IF and ACQ within TH. In VC, the ACQ improved at visit 2, but the IF did not.

Abstract P100 Table 1 Study groups and outcome measures

	Control (n = 12)	VC (n = 9)	TH (n = 9)
Sex (M/F)	7/5	4/5	7/2
Mean (SD) age, years	9.0 (2.0)	9.9 (3.3)	9.9 (1.3)
Mean (SD) FEV_1% predicted at visit 1	84.2 (19.6)	84.1 (13.9)	91.2 (14.6)
Median (quartiles) incorrect MDI steps at visit 1	2.0 (0; 4.75)	10 (6.5; 10)	6 (5; 9)
Median (quartiles) incorrect MDI steps at visit 2	0.5 (0; 2.75)	1.0 (0; 2.0)	0.0 (0; 0.5)
Mean (SD) peak IF pre-training at visit 1, l/min	46.7 (8.2)	99.1 (55.4)	115.8 (24.1)
Mean (SD) peak IF at visit 2, l/min	75.0 (34.2)	98.9 (65.8)	66.1 (19.0)
Mean (SD) ACQ at visit 1	1.14 (0.59)	2.43 (1.85)	2.39 (1.10)
Mean (SD) ACQ at visit 2	0.74 (0.93)	0.82 (0.64)	0.70 (0.97)

Conclusion VC and TH improved the children's MDI technique which was reflected on better asthma control. VC children could not, however, maintain the acceptable IF through their MDI which is critical for aerosol lung deposition. An inhaler training tool available to patients at any time can be helpful.

Best of science advances

P101 PERIPHERAL BLOOD TYPE 2 INNATE LYMPHOID CELL COUNT IN PATIENTS WITH SEVERE EOSINOPHILIC ASTHMA

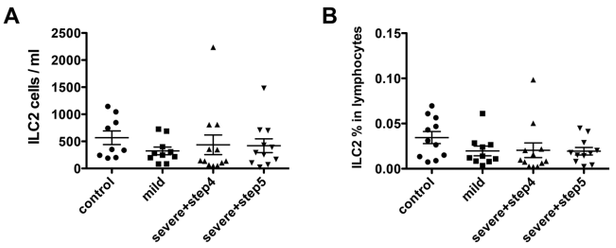
[1]B Hilvering, [2]S Go, [2]L Stoeger, [2]K Borg, [2]C Connelly, [2]S Thulborn, [2]S Pahlke, [2]ID Pavord, [2]L Xue. *[1]University of Utrecht, Utrecht, Netherlands; [2]University of Oxford, United Kingdom., Oxford, UK*

10.1136/thoraxjnl-2015-207770.238

Background A subgroup of patients with severe asthma have persistent eosinophilic airway inflammation despite treatment with high intensity corticosteroid treatment. One possible explanation for this pattern of disease is involvement of a type 2 innate lymphoid cells (ILC2s) dependant and relatively corticosteroid resistant pathway generating type 2 cytokines such as IL-5 and Il-13. The presence of high numbers of ILC2s in the nasal polyps commonly associated with severe eosinophilic asthma supports this view. We have carried out a cross-sectional study testing the hypothesis that ILC2 counts are increased in peripheral blood of patients with severe eosinophilic asthma.

Methods Blood was taken from 9 controls and 33 patients with asthma, 23 of whom met the 2014 ERS/ATS guideline criteria for severe asthma and had historical evidence of eosinophilic airway inflammation as defined before (Pavord *et al*. Lancet 2012;380:651–9). ILC2 were measured as lineage-$CD45^+CD127^+CRTH2+$ by flow cytometry and numbers presented as total cell counts and% peripheral blood mononuclear cells.

Results ILC2 counts were repeatable within patients (ICC 0.97; n = 6). Mean ± SD ILC2 counts were 566 ± 379, 323 ± 224, 437 ± 628 and 429 ± 421cells/mL (Figure 1A) and 0.034 ± 0.022, 0.02 ± 0.017, 0.020 ± 0.028 and 0.019 ± 0.014% of total lymphocytes (Figure 1B) in normal controls (n = 9), patients with mild to moderate asthma (n = 10), patients with severe asthma at BTS step 4 (n = 12), and patients with severe asthma at BTS step 5 (n = 11) respectively.



Abstract P101 Figure 1 Comparison of ILC2 counts (A) and proportions in lymphocytes (B) in the blood from healthy control and different asthma patients. ($p = 0.7$ for A and $p = 0.28$ for B)

Conclusion Type 2 innate lymphoid cells are scarce in peripheral blood but can be measured consistently. We found no evidence of increased counts in peripheral blood from patients with severe eosinophilic asthma.

P102 DEVELOPMENT OF A NOVEL ASSAY FOR THE DETECTION OF ACTIVE NEUTROPHIL ELASTASE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

[1]KL Moffitt, [1]SL Martin, [2]J Chalmers, [3]B Walker. *[1]Queen's University Belfast, Belfast, UK; [2]Ninewells Hospital and Medical School, Dundee, UK; [3]ProAxsis Ltd, Belfast, UK*

10.1136/thoraxjnl-2015-207770.239

Neutrophil elastase (NE), a biomarker of infection and inflammation, correlates with the severity of several respiratory diseases including chronic obstructive pulmonary disease (COPD). However, it's detection and quantification in biological samples is confounded by a lack of reliable and robust methodologies. Standard assays using chromogenic or fluorogenic substrates are not specific when added to complex clinical samples containing multiple proteolytic and hydrolytic enzymes which have the ability to hydrolyse the substrate, thereby resulting in an over-estimation of the target protease. Furthermore, ELISA systems measure total protease levels which can be a mixture of latent, active and protease-inhibitor complexes. Therefore, we have developed a novel immunoassay (ProteaseTag™ Active NE Immunoassay) which is selective and specific for the capture of **active** NE in sputum and Bronchoalveolar Lavage (BAL) in patients with COPD.

The objective of this study was to clinically validate ProteaseTag™ Active NE Ultra Immunoassay for the detection of NE in sputum from COPD patients.

20 matched sputum sol samples were collected from 10 COPD patients (M = 6, F = 4; 73 ± 6 years) during stable and exacerbation phases. Samples were assayed for NE activity utilising both ProteaseTag™ Active NE Ultra Immunoassay and a fluorogenic substrate-based kinetic activity assay.

Both assays detected elevated levels of NE in the majority of patients (n = 7) during an exacerbation (mean = 217.2 µg/ml ±296.6) compared to their stable phase (mean = 92.37 µg/ml ±259.8). However, statistical analysis did not show this difference to be significant ($p = 0.07$, ProteaseTag™ Active NE Ultra Immunoassay; $p = 0.06$ kinetic assay), which is highly likely to be due to the low study number. A highly significant correlation was found between the 2 assay types ($p \leq 0.0001$, $r = 0.996$).

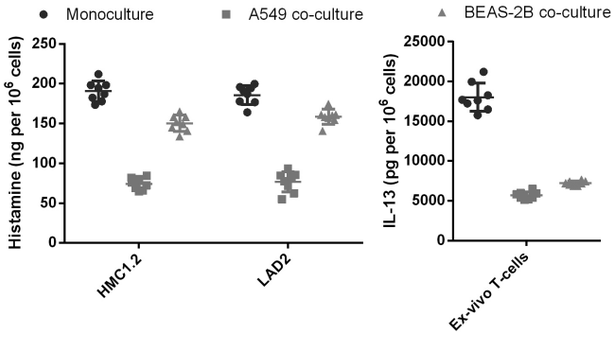
NE as a primary efficacy endpoint in clinical trials or as a marker of inflammation within the clinic has been hampered by the lack of a robust and simple to use assay. ProteaseTag™ Active NE Immunoassay specifically measures only **active** NE in clinical samples, is quick and easy to use (<3 h) and has no dependency on a kinetic readout. ProteaseTag™ technology is currently being transferred to a lateral flow device for use at Point of Care.

P103 INHIBITION OF ASTHMA-RELATED IMMUNOLOGICAL RESPONSES BY CULTURED EPITHELIAL CELL LINES

W Sargent, L Stoeger, I Pavord, TJ Powell. *Respiratory Medicine Unit, NDM, University of Oxford, Oxford, UK*

10.1136/thoraxjnl-2015-207770.240

Background Previous studies have shown that constitutive and IgE-mediated histamine production by human lung mast cells is inhibited by a transferable factor produced by the airway epithelium. We have tested the hypothesis that a similar interaction exists between epithelial cell and mast cell lines. We have also investigated the effect of co-culture of epithelial cell lines and T_H2 cells on interleukin (IL)-13 production.
Methods A549 or BEAS-2B cells were grown to confluence overnight. Media was removed and LAD2, HMC1.2 or human-derived ex-vivo T-cells added for 16 h. For transwell experiments epithelial cells were added to a 24-well plate, replaced with fresh media after 16 h and mast cells media added to the insert, maintaining the mast cell/epithelium/volume ratio. Wells, and transwell insert media, were then centrifuged, supernatants harvested and mediator release quantified by histamine or IL-13 ELISA.
Results Neither mast cell line consistently produced histamine in response to IgE and anti-IgE. Flow cytometry suggested that this was due to absence of the high-affinity IgE receptor FcεR1. Constitutive histamine production by HMC1.2 was reduced from 191 ± 13 ng/10^6 cells by 60.9% (95% CI 54.1, 67.8; $p < 0.0001$) when co-cultured with A549 and 21% (95% CI 14.2, 28.1; $p < 0.0001$) with BEAS-2B cells. Similar findings were seen with the LAD2 mast cell line. Constitutive IL-13 production by T_H2 cells was reduced from 18000 ± 1800 pg/10^6 cells by 68.6% (95% CI 62.0, 75.1; $P < 0.0001$) by A549 and 59.9% (95% CI 53.3, 66.5; $p < 0.0001$) by BEAS-2B. Epithelial inhibition was similar when cells were separated by a transwell suggesting involvement of a transferable factor.



Abstract P103 Figure 1 -hour constitutive histamine (left) and IL-13 (right) release from mast or T-cells in the presence or absence of epithelial cell lines

Conclusion Epithelial cell lines inhibit a range of asthma-related immunological responses, probably by producing an inhibitory substance.

P104 STRUCTURAL AND CELLULAR RELATIONSHIPS IN THE PERIPHERAL LUNG: COMBINING MICRO-CT AND IMMUNOHISTOCHEMISTRY

JJ Ramsden, JL Norman, PM Lackie, JA Warner. *University of Southampton, Southampton, UK*

10.1136/thoraxjnl-2015-207770.241

Introduction and objectives The peripheral lung contains a range of structural elements (small airways, blood vessels and lymphatics) together with infiltrating inflammatory cells. These components exist together in complicated spatial arrangements. Lung disease is frequently accompanied by changes in both lung architecture and the number and distribution of inflammatory cells. Light microscopy has been the conventional technique of choice in understanding these changes and relationships but provides only 2-D representations of a complex 3-D network.

We selected to use micro computed tomography (μ-CT) to image structural elements in the peripheral lung. We aimed to reconstruct the 3-D architecture by combining the μ-CT data with immunohistochemistry (IHC) to positively identify the principal structural elements and inflammatory cells.
Methods Human lung tissue was fixed in formalin, embedded in paraffin wax and subjected to μ-CT scanning. The tissue was then sectioned and immunostained for pancytokeratin (airways), collagen IV (blood vessels), D2–40 (lymphatic vessels) and CD68 (macrophages). The resulting images were used to guide the segmentation of the 3-D μ-CT image stack. IHC, using neurofilament antibodies, was also used on multiple lung samples to attempt to identify nerve fibres in the parenchymal tissue.
Results The main structural elements of the lung periphery could be identified, segmented out and their 3-D architecture examined. Macrophages were found throughout the tissue in large quantities and were most concentrated around the blood vessels and lymphatics. Lymphatic vessels were especially dense in the pleural region and elsewhere were intertwined with blood vessels. Despite being readily identifiable in bronchial samples, nerve fibres were not identified using IHC in the parenchyma.
Conclusions Combining μ-CT and IHC provides a robust method to positively identify important structural elements of the peripheral lung and to localise inflammatory cells in 3-D, thus allowing a detailed review of their spatial relationships. Alternative methodologies may however be advantageous regarding identifying parenchymal nerve fibres for reconstruction. μ-CT and IHC together create a highly accurate 3-D reconstruction but this method remains time consuming; advances in automation and improved tools are required to fully exploit the research potential.

P105 IDENTIFICATION OF 'LARGE' ALVEOLAR MACROPHAGES AND PULMONARY INTRA-VASCULAR MACROPHAGES IN COPD PATIENTS

AK Ravi, J Plumb, S Mason, G Booth, J Vestbo, SD Singh. *The University of Manchester, Manchester Academic Health Science Centre, University Hospital of South Manchester NHS Foundation Trust, NIHR South Manchester Respiratory and Allergy Clinical Research Facility, Manchester, UK*

10.1136/thoraxjnl-2015-207770.242

Background A population of small macrophages with increased pro-inflammatory activity has been reported in COPD sputum. We have investigated macrophage size in the alveoli of COPD