Blood and BAL eosinophils had the strongest correlation (r = 0.57, p < 0.001, n = 84). Weaker correlations were found between the other measures. The most promising predictor of BAL eosinophilia was a blood eosinophil count of 0.15 × 10^9/L (PPV 84.1, NPV 71.4) (Table 1).

Conclusions These results suggest that blood eosinophils at a lower cut-point may be a useful measure of lower airway inflammation. However, this is still a relatively invasive test in children and there is little data available about longitudinal stability of blood eosinophils.

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P99 COLONISATION WITH FILAMENTOUS FUNGI AND ACUTE EXACERBATIONS IN CHILDREN WITH ASTHMA

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10.1136/thoraxjnl-2015-207770.236

Background Children with asthma are frequently sensitised to fungi and recent observations suggest that fungal sensitisation may be associated with more severe asthma in children.[1,2] *Aspergillus* fumigatus airway colonisation in adults with asthma is associated with reduced lung function.[3] There is a paucity of data onfungal colonisation in children with asthma. The role of fungi in exacerbation prone asthma has not been previously investigated. Our study aim was to evaluate the association between fungal airway colonisation and exacerbation prone asthma in children.

Methods Children aged 5–16 years with stable asthma who attended for a routine hospital outpatient appointment and children with an acute exacerbation of asthma who attended for urgent care to an acute admissions unit were recruited to the study. We obtained a sputum sample either via nebulisation with hypertonic saline in children with stable asthma or nebulisation with 0.9% saline in children with acute asthma. Sputum culture was focused to detect filamentous fungi, in particular *Apergillus* and *Penicillium* species.[3,4] Culture and sensitisation results were compared with clinical assessment data.

Abstract P99 Table 1 Demographics and fungi isolated in acute and sasthma

	Acute asthma n = 26	Stable asthma n = 29	P value*
Median age in years (range)	8 (5–15)	10.5 (5–16)	0.122
Male (%)	17 (65.4)	20 (68.9)	0.778
Filamentous fungi culture positive n (%)	11 (42.3)	5 (17.3)	0.041
A. fumigatus	9	4	
A niger	0	1	
Penicillium	2	1	

*Mann Whitney or Chi squared test.

Results Fifty five children were recruited to the study; 26 with acute asthma and 29 with stable asthma (17 BTS step 4–5). There was no difference in demographics between the two groups (Table 1). Sixteen children (29%) were culture positive for filamentous fungi, either *Aspergillus* fumigatus *(81.3%)* or *Penicillium (18.7%)*. One child with stable asthma harboured two different filamentous fungi, *A.* niger and *A* fumigatus. Children with acute asthma were more likely to be culture positive for filamentous fungi than children with stable asthma (42.3%, n = 11 v 17.2%, n = 5 respectively, p = 0.041). Of the five children with stable asthma who were culture positive for filamentous fungi, three were BTS step 4–5.

Conclusions Significantly more children with acute asthma had filamentous fungi isolated from their sputum compared to children with stable asthma. *Aspergillus fumigatus* was the most common fungus isolated. The potential role of fungal airway colonisation in triggering asthma attacks in children merits further investigation.

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P100 IMPROVING PAEDIATRICS' PRESSURISED METERED DOSE INHALER TECHNIQUE AND ASTHMA CONTROL: INHALER VERBAL COUNSELLING VS. TRAINHALER

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10.1136/thoraxjnl-2015-207770.237

Introduction Verbal counselling (VC) is commonly used to train patients on correct inhaler technique. Patients forget the good inhaler use with time. Trainhaler (TH), Clement Clarke, UK, is a novel pressurised metered dose inhaler (MDI) training tool designed with feedback mechanisms to train patients coordinate releasing the aerosol with using a slow and deep inhalation flow (IF) through their MDI. Our aim was to compare VC with TH in children with asthma.

Methods Ethical approval was obtained and all children and their parents gave signed consent. At visit 1, asthmatic children, age 7–17 years, with an MDI hand-lung coordination problem including an IF >60 l/min were randomised into either the VC group that received verbal MDI training with emphasis on using a slow and deep IF; or into the TH group that were trained on-and given TH to practice at home. Children with correct MDI technique and IF ≤60 l/min formed the control group (CT). An 11-step MDI technique, peak IF through the inhaler and Asthma Control Questionnaire (ACQ) were evaluated. All subjects returned after 6 to 8 weeks (visit 2) for re-evaluation.

Results Thirty children took part. Table 1 presents the study outcomes. All VC and TH had correct MDI steps and slow IF post-training at visit 1. Unlike CT, Wilcoxon test showed a significant decrease (p < 0.01) in the incorrect MDI steps between visits 1 and 2, within VC and TH. Mann-Whitney test showed a significant difference (p < 0.01) in the incorrect MDI steps between the CT and both intervention groups at visit 1, but no significant difference (p > 0.05) was found at visit 2. Paired t-test showed

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

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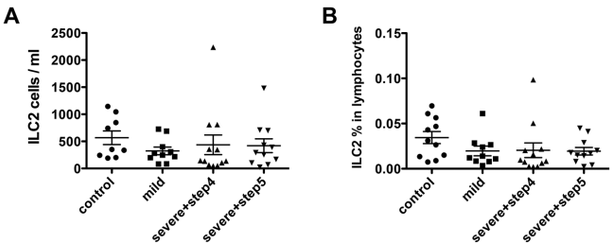
10.1136/thoraxjnl-2015-207770.238

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

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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 ($p \leq 0.0001$, $r = 0.996$).