# 491. Severe asthma and developing allergy in childhood

**4708**

**Bronchoscopy and endobronchial biopsies in children: Useful or not?**

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**Introduction:** Bronchoscopy and endobronchial biopsies are diagnostic tools in the evaluation of difficult asthma and other paediatric airway diseases. The yield of these procedures in clinical practice is uncertain. Therefore our aim was to evaluate if bronchoscopy and endobronchial biopsies change management.
**Methods:** Retrospectively we collected data on diagnosis and treatment before and after bronchoscopy in all children undergoing bronchoscopy with endobronchial biopsies between 0 and 18 yrs. Difficult asthma was defined as uncontrolled asthma despite high dose ICS dose and LABA. In endobronchial biopsies reticular basement membrane (RBM) thickness was measured.
**Results:** Of the 74 children (37 male, mean age 7.8 yrs), 26 had asthma and 13 had difficult asthma. The diagnosis of children undergoing bronchoscopy changed in 31.3% of the cases: 10 children got a second diagnosis, which could explain the persistent symptoms, 8 children were misdiagnosed. In these 18 children airwaymalacia (n=10) and anatomic abnormalities (n=7) were the most common. In 9.5% of the cases biopsies changed the diagnosis; in 3 of them ciliary dyskinesia was suspected. In 25% medication was changed after bronchoscopy.
Children without asthma had a RBM of 5,6 μm [SD ±1,4]; in children with asthma RBM thickness was 5,11 μm [SD ±1,05] and in those with difficult asthma 6,0 μm [SD ±1,34] (p=0.49).
**Conclusion:** Bronchoscopy showed an important role to reveal underlying causes of persistent symptoms such as airwaymalacia and anatomic abnormalities. In 25% of patients bronchoscopy led to a change in treatment. We found no significant difference in RBM thickness between nonasthmatic, asthmatic children and children with difficult asthma.

**4709**

**IL-33 and airway remodelling in paediatric severe therapy resistant asthma**

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**Background:** Associations have been reported between epithelial interleukin (IL)-33 expression and severe asthma in adults, but its role in the pathophysiology of paediatric severe therapy resistant asthma (STRA) remains unknown.
**Objectives:** We quantified IL-33 expression in endobronchial biopsies from children with STRA and explored its relationship with parameters of airway remodelling.
**Methods:** Children (n=45) (median age 11.3 [range 5.4-16.4] years) with STRA underwent detailed investigations including bronchoscopy and endobronchial biopsy. IL-33+ cells were quantified in the epithelium, smooth muscle and submucosa in biopsies from children with STRA and compared to that in age matched non-asthmatic controls. Reticular basement membrane (RBM) thickness was quantified as a marker of airway remodelling, and related to IL-33+ cells.
**Results:** Submucosal, but not epithelial or smooth muscle IL-33+ cells were significantly higher in children with STRA compared to controls (p=0.01, 0.21, 0.68 respectively).
In addition, there was a positive correlation between IL-33+ cells and RBM thickness in children with STRA (Spearman r=0.35, p=0.018), but not in controls (p=0.33).
**Conclusions:** In contrast to adult studies, we have shown increased submucosal IL-33 expression in children with STRA, and for the first time have shown an association between this mediator and airway remodelling. These data suggest therapies that block IL-33 may be suitable for paediatric STRA, and specifically may impact on structural airway changes.

**4710**

**Exhaled nitric oxide in children with severe asthma**

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**Introduction:** Exhaled nitric oxide ($FE_{NO}$) is a biomarker of eosinophilic airway inflammation, but the relationship between $FE_{NO}$ and asthma severity is uncertain. We compared $FE_{NO}$ normalized against reference values ($\%FE_{NO}$) in children with Problematic severe (PA) and controlled (CA) asthma and investigated whether increased $\%FE_{NO}$ is associated with morbidity, irrespective of predefined severity classification.
**Methods:** Children with PA had recurrent symptoms despite treatment with ≥800 μg Budesonide, those with CA had few symptoms with 100-400 μg. The protocol included Asthma control test, spirometry (%), methacholine provocation (dose response slope), $FE_{NO}$ (p.p.b.), computerized tomography (CT) of the lungs (PA only) and blood sampling for eosinophils ($10^9$ x $L^{-1}$) and IgE (kU/L). The difference between measured and expected $FE_{NO}$ ($Ln(FE_{NO})$ = 0.0112 x height (cm) + 0.641) were given in percentages ($\%FE_{NO}$).
**Results:** Children with PA (n=57, age 13y) had a trend towards higher levels of $FE_{NO}$ and $\%FE_{NO}$ compared to children with CA (n=39, age 14y): 22 (10-40) vs. 17 (9-26), p=0.13 and 210% (101-367) vs. 139% (85-216), p=0.07, respectively. When analysing all children (n=96), those with $\%FE_{NO}$>200 had reduced asthma control (18.5 (17-20) vs. 20.4 (19-22), p=0.04) and FEV1/FVC (77 (74-81) vs. 83 (81-86), p=0.004) and increased bronchial hyperresponsiveness (54 (5-67) vs. 2 (0.4-36), p=0.001), bronchial wall thickening on CT (25 (21-29) vs. 17 (14-19), p=0.004), eosinophils (0.5 (0.4-0.6) vs. 0.3 (0.2-0.3), p<0.001) and IgE (539 (253-1525) vs. 140 (43-425), p<0.001) compared to those with $\%FE_{NO}$<200.
**Conclusion:** Children with high levels of $\%FE_{NO}$ have increased morbidity which is partly independent of predefined severity classification.

**4711**

**Fungal sensitisation in children with severe therapy resistant asthma**

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Adults with severe asthma with fungal sensitisation (SAFS) have reduced lung function and increased morbidity [Am J Respir Care Med 2009;179:11-8]. We hypothesized that fungal sensitisation in children with severe, therapy-resistant asthma (STRA) is associated with increased morbidity.
**Methods:** All children had STRA having had basic management optimized [Lancet 2010;376:814-25]. There were 166 patients (11.7 years [4-17]; 61% boys). SAFS (n=76) was defined as specific IgE (spIgE) or skin prick test (SPT) positivity to Aspergillus fumigatus, Alternaria alternata or Cladosporium herbarum. Non-sensitised patients (n=90) had negative spIgE and SPT. Age, atopy, symptoms, medication, lung function and airway inflammation were assessed.
**Results:** SAFS children were mainly boys (57/76(75%) vs 43/90(48%), p<0.001), had earlier asthma onset (0.5 years [0-12.5] vs 1.5 [0-12.5], p=0.006), higher total IgE (637 IU/mL [12-6737] vs 177 [1-10881], p=0.002) and sum of inhalant allergen SPT and spIgE (16 mm [0-38] vs 9 [0-36], p<0.001; 78 IU/mL [0-400] vs 19 [0-243], p=0.02). SAFS children had a trend for lower FEV1 (72%Pred [29-121] vs 75.5 [23-125], p=0.18) and FVC (90%Pred [36-138] vs 95 [30-123] p=0.13), more bronchodilator reversibility (59/73 (81%) vs 42/81 (52%), p<0.001), and were more likely prescribed maintenance oral steroids (18/76 (24%) vs 8/88 (9%), p=0.02). Symptoms and airway inflammation (sputum, bronchoalveolar lavage and endobronchial biopsy) were similar.
**Conclusions:** Children with STRA and SAFS had earlier asthma onset, more atopy and bronchodilator reversibility, and were more often given prednisolone. We need a randomised controlled trial of antifungal therapy in paediatric SAFS.

**4712**

**Urinary eicosanoids and preschool wheeze phenotype**

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**Rationale:** Preschool wheeze follows two broad clinical patterns; i) multi-trigger wheeze (wheeze both with and between colds) and ii) episodic viral wheeze (wheeze exclusively with colds). Whether these patterns reflect stable, distinct phenotypes with different underlying inflammation remains unclear.
**Hypothesis:** We hypothesised that preschool children with multi-trigger wheeze have chronic pulmonary leukotriene activation, and therefore higher baseline urinary eicosanoids, than episodic viral wheezers.
**Methods:** Children aged 10 months to 5 years with wheeze and no respiratory comorbidity were recruited from three UK hospitals. Wheezing pattern was obtained from parental history. Urine was collected between viral-triggered attacks and frozen at -70°C. Samples were analysed using high performance liquid chromatography tandem mass spectrometry for a range of eicosanoids (Table 1).
**Results:** See Table 1. There was no significant demographic difference between multi-triggered (n=46) and episodic (n=115) wheezers. There was no significant difference in baseline eicosanoid excretion between the 2 groups (P=NS by unpaired t-test, Table 1).



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Table 1. Baseline urinary eicosanoids (pg/mg of creatinine)

| | Episodic | Multitrigger |
|---|---|---|
| Leukotriene E4 | 1216.47 | 928.71 |
| 13,14-dihydro-15-keto-PGE2 | 455.03 | 625.38 |
| 13,14-dihydro-15-keto-PGD2 | 198.32 | 245.59 |
| 13,14-dihydro-15-keto-tetranor-PGE2 | 1281.95 | 1837.72 |
| 13,14-dihydro-15-keto-tetranor-PGD2 | 2108.84 | 1845.95 |
| tetranor-PGE-M | 35655.09 | 34995.67 |
| tetranor-PGD-M | 45810.02 | 43065.58 |
| 15-deoxy-delta12,14-PGJ2 | 361.61 | 606.20 |
| 9a,11b-PGF2 | 695.37 | 872.18 |

**Conclusions:** We found no evidence that the multi-trigger phenotype is associated with increased baseline eicosanoid activation.
Dedicated to the memory of Prof Andrzej Szczeklik who would have been a co-author.

**4713**

**Changes in skin prick test (SPT) from the age of 1 to 6 years (yrs) and relationship to specific IgE and atopic dermatitis: The French arm of PASTURE European study**

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The "PASTURE" study (Protection against Allergy: STUdy of Rural Environment) examines the influence of exposure to a dairy-farm environment on the occurrence of allergy in a cohort of European children from the 3rd trimester of pregnancy to 10-yrs.
The aim of this study was to analyze the course of SPTs to aero- and food allergens from the age of 1 to 6 yrs in the french children of PASTURE, whether they live or not on a farm and to evaluate their relationship to specific IgE and atopic dermatitis (AD).
Two hundred and four french children, which 95 of the farmer group, were included. SPTs, AD assessment and specific IgE assays were performed at 1, 4.5 and 6 yrs.
One hundred and forty children participated in all three visits. The prevalence of positive SPTs increased with age (9.5% at 1 yr, 14.2% at 4.5 yrs, 22.6% à 6 yrs). It was lower in the farmer group, regardless of age and category of allergens considered (statistically significant only for seasonal allergens at 4.5 yrs). Positive SPTs were transient at 1 yr whereas they were persistent between 4.5 and 6 yrs. The prevalence of AD was not significantly different in the two groups and decreased with age. Positive SPTs at 1 yr were predictive of the occurrence of AD during the follow-up. The correlation between SPTs and specific IgE was poor for an IgE cut-off at 0.35UI/mL but increased with age and with higher cut-off.
In conclusion, our study showed that skin reactivity (1) increased with age, (2) was lower in the farmer group, (3) at 1 yr was transient but predictive of the occurrence of AD. Correlation between SPTs and specific IgE was poor, especially at one yr.

**4714**

**DNA methylation in asthma and allergy related genes is influenced by farm exposure and time trends in early childhood**

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Genes and environmental influences are crucial for the development of asthma and atopic diseases. These factors may act upon each other through epigenetic mechanisms.
We studied the rural birth cohort PASTURE (Protection against allergy: study in rural environments) to investigate if (a) epigenetic patterns in asthma candidate genes are influenced by farm exposure in general and (b) change over the first years of life and (c) whether these changes may contribute to the development of asthma.
DNA was extracted from cord blood and whole blood at age 4.5 in 48 samples per time point. We analyzed DNA methylation of 23 regions in ten asthma and allergy candidate genes (*ORMDL1*, *ORMDL2*, *ORMDL3*, *CHI3L1*, *RAD50*, *IL13, IL4*, *STAT6*, *FOXP3* and *RUNX3*) by pyrosequencing and compared differences between strata.
In cord blood, regions in *ORMDL1* and *STAT6* showed a hypomethylation while regions in *RAD50* and *IL13* were hypermethylated when DNA from farmers was compared to non-farmers (lowest p-value 0.001 for *STAT6*). Only small associations between asthma status and methylation were observed in our population. Changes in methylation over time occurred in 14 out of 23 gene regions investigated (lowest p-value for *IL13*, $p=1.57*10^{-8}$). Interestingly, these differences clustered in the genes highly associated with asthma (ORMDL family) and IgE regulation (*RAD50*, *IL13* and *IL4*) but not in the T-regulatory genes (*FOXP3*, *RUNX3*).
Our study indicates that DNA methylation changes significantly in early childhood in specific asthma and allergy related genes in peripheral blood cells while also early exposure to farm life seems to influence methylation patterns in certain genes.

**4715**

**Does breastfeeding influence development of atopy? A cohort study**

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**Aim:** It has been postulated that breastfeeding (BF) reduces the risk of allergy in children. However, recent studies failed to show a protective effect or suggested an increased risk of atopy with BF. We examined the association between BF and atopy, measured by skin prick test (SPT), in a large population-based cohort, stratifying by maternal hayfever.
**Method:** We assessed BF in the first years of life in children from Leicestershire, UK. At school age (9-14 years), we performed SPT for 4 allergens (cat, dog, dust, grass) in 1515 children. A child was defined as atopic if any wheal size was >3mm. We used logistic regression, adjusting for age, sex, ethnicity, birth season, family history of atopy and socio-economic status. We stratified by maternal hayfever and investigated potential reverse causation in analysis that excluded children with eczema or wheeze in infancy.
**Results:** 465 (31%) children were not breastfed, 435 (29%) had BF <3 months (mo), 256 (17%) BF=4-6 mo and 348 (23%) BF >6 mo. 437 mothers (29%) had hayfever. We found no evidence for differences in risk of atopy by BF; the odds-ratios (95% confidence intervals) for BF<3 mo, BF=4-6 mo and BF>6 mo were 0.97 (0.69,1.37), 1.07 (0.72,1.59) and 1.19 (0.83,1.71), respectively, compared with no BF (p-trend=0.297). We did not find differential association by maternal hayfever (p-interaction=0.489) nor evidence for reverse causation.
**Conclusion:** Using objective measures and controlling for reverse causation, our study did not find evidence for an effect of breastfeeding on atopy in children of mothers either with or without hayfever. We can continue recommending breastfeeding for atopic and non-atopic mothers.
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