Does airway allergic inflammation pre-exist before late onset wheeze in children?

Epidemiological studies have shown that some children develop wheezing after 3 yr of age which tends to persist. It is unknown how this starts or whether there is a period of asymptomatic inflammation. The aim of this study is to determine whether lower airway allergic inflammation pre-exists in late onset childhood wheeze (LOCW). Follow-up study of children below 5 yr who had a non-bronchoscopic bronchoalveolar lavage (BAL) performed during elective surgery. The children had acted as normal controls. A modified ISAAC questionnaire was sent out at least 7 yr following the initial BAL, and this was used to ascertain whether any children had subsequently developed wheezing or other atopic disease (eczema, allergic rhinitis). Cellular and cytokine data from the original BAL were compared between those who never wheezed (NW) and those who had developed LOCW. Eighty-one normal non-asthmatic children were recruited with a median age of 3.2 yrs. Of the 65 children contactable, 9 (16.7%) had developed wheeze, 11 (18.5%) developed eczema and 14 (22.2%) developed hay fever. In five patients, wheeze symptoms developed mean 3.3- yr (range: 2–5 yr) post-BAL. Serum IgE and blood eosinophils were not different in the LOCW and NW, although the blood white cell count was lower in the LOCW group. The median BAL eosinophil % was significantly increased in the patients with LOCW (1.55%, IQR: 0.33 to 3.92) compared to the children who never wheezed, NW (0.1, IQR: 0.0 to 0.3, p = 0.01). No differences were detected for other cell types. There were no significant differences in BAL cytokine concentrations between children with LOCW and NW children. Before late onset childhood wheezing developed, we found evidence of elevated eosinophils in the airways. These data suggest pre-existent airways inflammation in childhood asthma some years before clinical presentation.

Epidemiological studies have shown that in the majority of young children with respiratory symptoms in the first few years of life, the wheezing usually resolves with increasing age (transient wheezers) (1, 2). In many children with true allergic asthma, wheezing and respiratory symptoms can be traced back to early childhood. However, Martinez et al. clearly described a cohort of children who did not report symptoms in the 1st 3 yr of life but subsequently reported wheezing at age 6. These children, labelled late onset childhood wheezers (LOCW), were likely to develop persistent symptoms of asthma.

Over the last 20 yr, we and others have shown that children with atopic asthma have significantly elevated levels of eosinophils in bronchoalveolar lavage (BAL) (3–5), while children...
who have viral associated wheeze have shown no evidence of chronic inflammation or have neutrophilia in their BAL (6–8). Recent evidence from bronchial biopsies in young children older than 2 yr with troublesome wheezing or asthma has shown that airway remodelling as indicated by reticular basement membrane thickening probably occurs early in childhood rather than being a consequence of long-term uncontrolled airways inflammation (9, 10). Pohunek et al. (11) showed evidence of chronic inflammatory cell infiltrates and airway remodelling in the lower airway of preschool children before a diagnosis of asthma had been made; however, these children had already presented with recurrent respiratory symptoms.

Longitudinal studies suggest that there is a loss of airway function associated with early childhood asthma (12). Extrapolating from adult disease and the few tissue-based studies of children, this would appear to be related to abnormal postnatal development or remodelling of the airway walls. Little is known as to the pathophysiological sequence of events leading to wheezing illnesses in childhood and especially for the development of true persistent asthma. Early childhood may be an important time when disease-modifying or preventative therapies could be applied before the persistent asthma phenotype becomes established. Disappointingly, inhaled corticosteroids given for 2 yr to high-risk infants and young children reduced current wheezing but did not have longer-term disease-modifying effects (13, 14). The onset of asthma in children whose first wheezing episode occurred after 3 yr of life could have resulted from a ‘hit and run’ effect triggered by a viral infection. Alternatively, such children may have experienced a subclinical period of allergic airways inflammation occurring before the onset of symptoms.

To provide further information regarding the ontogeny of allergic inflammation in LOCW, we have followed up a group of children who had a BAL sample obtained when they were less than 5 yr of age, and at the time of the BAL, sample had been reported to have never wheezed. We anticipated that some of these children would have developed late onset wheezing.

**Aim**

To address the poorly understood issue of origins of airway inflammation in childhood asthma by determining whether lower airway allergic inflammation pre-exists in children before late onset of wheeze.

### Materials and methods

**Patients**

The patients included in this analysis are children (<5 yr) who were the normal controls in our previous research (non-bronchoscopic BAL) (4, 6, 8, 15–17). These children were undergoing elective surgery for non-pulmonary illness at the Royal Belfast Hospital for Sick Children. Parents were asked questions to determine the atopic status of the child and in the family which included wheeze, allergic rhinitis, eczema and ‘other allergies’. These children were reported never to wheeze or had eczema or allergic rhinitis; none were receiving medication and had any recent history of acute respiratory infection at the time of investigation. A modified ISAAC questionnaire (8) was posted out to parents at least 7 yr post the initial BAL to ascertain how many children had subsequently developed wheeze and other atopic disorders. Non-returners were sent a 2nd postal questionnaire, and if this was not returned, an attempt to contact the parents was made by telephone, and the researcher (GW) ‘read out’ the questions over the telephone.

In addition, we recorded which children had developed other atopic disorders (allergic rhinitis and eczema). Patients were classified as late onset childhood wheezers (LOCW), non-wheezers (NW) or non-wheezers with other atopy (NWA). The initial studies were approved by Queen’s University Belfast Research Ethics Committee, and this follow-up study was approved by Office of Research Ethics Committee Northern Ireland (ORECNI).

**Sampling and processing of Bronchoalveolar Lavage**

A blind non-bronchoscopic BAL was performed under general anaesthesia (18). Briefly, following endotracheal intubation (ET), an eight French Gauge feeding tube was inserted blindly through the ET tube and wedged in a distal bronchus. Twenty millilitres normal saline (warmed to body temperature) was instilled and immediately withdrawn by gentle manual suction. At the same time, a venous blood sample was taken, and serum IgE, total blood white cells and eosinophil counts were analysed in the routine clinical fashion.

Cell-free supernatants were obtained by centrifugation (400 g, 5 min, 4°C) and stored in the presence of a protease inhibitor cocktail. Total cell counts were determined with a haemocytometer. Viability was assessed by the trypan blue exclusion test. Cytospin preparations produced
using a modified coverslip method (18). Cells were stained with Diff-Quick® (Baxter Healthcare Ltd, Compton, UK) for differential cell counting (minimum of 500 cells counted).

Detection of BAL fluid cytokine concentrations was performed using enzyme-linked immunosorbent assay (ELISA) for EGF analysis and Bio-Plex Human Cytokine Th1/Th2 Panel (Bio-Rad 171-A11081) for the other cytokines.

Statistics
As some data were from a skewed distribution, the results are expressed as median with interquartile range (IQR) or mean (SD) when appropriate. Comparisons between groups were made by the Mann–Whitney U test for unpaired samples. In situations where many of the BAL cytokine concentrations were tied values and below the detection limit, the Median test was used for between group comparisons. We compared the % BAL eosinophil between LOCW, NW and NWA using a Kruskal–Wallis test. We assessed how useful peripheral blood eosinophil percentage (%) was at predicting subsequent LOCW (Yes/No) and any atopy (Yes/No) by way of sensitivity, specificity and area under the receiver operator curves, AUC and ROC. A two-tailed p-value of equal to or < 0.05 was considered statistically significant.

Results
Eighty-one children <5 yr who had been normal controls recruited into our previous studies were identified. These children, at the time of BAL sampling, had no reported wheezing, eczema, allergic rhinitis or other allergies. Sixteen children were not contactable. Twenty-two children had developed symptoms of wheezing or other atopic diseases (eczema and/or allergic rhinitis). Nine children (17%) had developed LOCW, 11 children developed eczema and 14 children had hay fever symptoms (Fig. 1). All the children with LOCW had been diagnosed by a doctor as having asthma, and most were on regular inhaled corticosteroid and intermittent short acting beta-agonist therapy. Six of the nine children had a family history of asthma and/or other atopic history.

Of the 65 children contactable, 62 children had adequate BAL return to obtain cellular data (8 LOCW and 54 NW) and 54 children (9 LOCW and 45 NW) had adequate BAL return for cytokine analysis. These children [mean age 3.2 (range: 1–4.8) at the time of elective surgery had no history of atopic disorders and had a normal serum IgE measurement [LOCW: 12 (7–28) IU/ml vs. NW: 12 (5–28) IU/ml]. Data from only five of the 8 LOCW reporting the subsequent development of wheeze seemed of sufficient precision to allow calculation of the time elapsed between the initial BAL sampling and the subsequent first wheezing episode. For these 5 LOCW, the onset of symptoms developed a mean of 3.3 yr (range 2–5 yr) post the initial BAL.

The blood white cell count in the LOCW (median 6.04 × 10⁵, IQR: 5.4–7.5) was significantly lower than in the NW (median 8.40 × 10⁵, IQR: 6.9–10.5), p = 0.03. Interestingly, although the blood eosinophils % available for 7 LOCW children (median 8.5, IQR: 1.7–10.2) appeared higher compared to the 11 NWA (median 2.43, IQR 2–6.2) and 35 NW children (median 2.65, IQR: 2–4.4), this was not statistically significant (p = 0.27) (Fig. 2). ROC analysis of blood eosinophils for the prediction of any subsequent atopy (Yes/No) using a blood eosinophils >5% as the best cut-off showed an AUC of 0.57, sensitivity of 44% and specificity of 89%. Similarly, ROC analysis of blood eosinophils using an eosinophil count % >7.8% as the best cut-off for LOCW showed an AUC of 0.63, sensitivity of 57% and specificity of 94%.

Details of the different BAL cell types in NW and LOCW children are shown in Table 1. There were similar numbers of total cells, percentage macrophages, lymphocytes and neutrophils between BAL LOCW and NW (Table 1). However, BAL eosinophil counts were significantly

![Venn Diagram describing the different overlapping patient groups, late onset childhood wheeze, eczema and allergic rhinitis.](image-url)
increased in children with LOCW (median 1.55, IQR: 0.33–3.92) compared to the NW children (median 0.10, IQR: 0.0–0.3, p = 0.01). Including NWA in the analysis and comparing with LOCW and NW, there was a significant increase in BAL eosinophil percentage only in children with LOCW (Fig. 3). In the LOCW group, two children had 0% BAL eosinophils. The first child was a boy, with a low IgE (2 ku/l) and a blood eosinophil of 2.2%. The second child was a girl and also had a low IgE (12 ku/l) but did have an elevated % blood eosinophil (10.2%).

**BAL Cytokine data**

Of the 65 children contactable, an adequate volume of BAL for cytokine analysis was available for 54 patients (9 LOCW and 45 NW). There were no differences in BAL cytokines concentrations between LOCW and NW (Table 2).

**Discussion**

The presence of raised BAL eosinophil percentages in airways of pre-symptomatic children who subsequently developed asthma has not been documented previously. Our results show that there is evidence of a significant early eosinophil inflammation prior to the onset of asthma symptoms, suggesting that a ‘mechanism’ has been ‘switched on’ in the naive airways that leads to an asthmatic phenotype.

This finding is timely and relevant because a variety of clinical and epidemiological studies strongly suggest that in asthma, remodelling may start very early in life (19) and current prevention and treatment measures, including early avoidance measures and pharmaceutical interventions, are relatively ineffective in preventing the development of irreversible airway changes or in reversing them, once established (20, 21).

The finding that eosinophils in BAL are elevated in some children who subsequently developed asthma would suggest other components of inflammation may be present. Infection prior to surgery in the lower airway would manifest with a neutrophilia (22), and this study does not demonstrate any difference in BAL neutrophil percentage in the children who subsequently developed asthma compared with normal controls. The increase in % BAL eosinophil was not mirrored by elevated cytokines as observed in children with established asthma during a quiescent phase (23). Of the nine children with subsequent LOCW, six had a positive family history of atopy, a marker of potential development of asthma (21).

![Blood Eosinophils % counts of the different clinical subgroups.](image)  
**Fig. 2.** Blood Eosinophils % counts of the different clinical subgroups. There was no significant increase in blood eosinophils in the late onset childhood wheeze when compared with children who never wheezed or children who had subsequently developed atopy but never wheezed.

![Bronchoalveolar Lavage (%) Eosinophils of the different clinical subgroups.](image)  
**Fig. 3.** Bronchoalveolar Lavage (%) Eosinophils of the different clinical subgroups. There was a significant increase in bronchoalveolar lavage % eosinophils in the late onset childhood wheeze when compared with children who never wheezed or children who had subsequently developed atopy but never wheezed.
Currently, there are no diagnostic markers of bronchial asthma, which, on their own, either confirm or exclude asthma with appropriate sensitivity and specificity. Although an elevated blood eosinophil % measured in the first few years of life (when children with LOCW were asymptomatic) was reasonable specific for the prediction of LOCW or other atopy, it had a low sensitivity. Clinically, the most reliable feature of asthma that seems to be related closely to the symptoms is the presence of eosinophils in bronchoalveolar lavage (4, 8, 15).

Unfortunately, we do not have lung function data on our group of children. Baldwin et al. (12) demonstrated evidence of loss of airway function in early childhood asthma because of persistent airway inflammation in the absence of clinical symptoms. This worrying observation suggests that airway inflammation and early remodelling may occur in a subclinical disease state (24). There is also accumulating evidence that subsequent lung function is ‘set’ in the first years of life (25). Early life events can lead to the development of a Th2-like environment, which is influenced by infections (Th1) rather than allergen exposures, diet and certain respiratory infections. The tendency towards a Th2-like setting will promote the likelihood of IgE production towards environmental allergens. With sensitization, there can be the development of acute airway inflammation, then persistent inflammation and eventually airway injury and remodelling (26).

It is important to try to identify which factors are associated with airway inflammation in children who subsequently developed late onset asthma. It was suggested that children with wheezing of late onset were significantly more likely than those who had never wheezed to have mothers with asthma, to be male, and to have had rhinitis in the first year of life (1). This study clearly has shown that atopy cannot predict eosinophilic airway inflammation as there were no significant differences in blood and BAL eosinophils and serum IgE between the three different subgroups. These findings have also been seen both in adult and paediatric studies (5, 27).

A deficiency of this study and also with many paediatric studies on asthma is the known inaccuracy of parental reporting of symptoms and especially of wheezing (22). Another limitation of this study includes question as to how samples of BAL fluid represent true airway pathology. At a single time-point, airway inflammation may exist only in the mucosa and submucosa and not be found in the airway lumen. Therefore, when performing a blind nonbronchoscopic BAL, samples obtained represent only a small segment of the total airway which may or may not contain any inflammatory cells, which may provide a false negative result. A non-invasive and specific marker of eosinophilic airway inflammation would be a valuable tool to study this, and exhaled nitric oxide or induced sputum may be useful in this regard.

In an ideal situation, it would be good to recruit a cohort of children from infancy (with or without an atopic history) and have them followed up prospectively; having BAL and bronchial biopsies performed at regular intervals to measure inflammatory cells and pulmonary function tests carried out to identify ‘at-risk’ patients who subsequently developed asthma; however, for ethical reasons this is not possible.

The frequency of LOCW reflects quite well the population frequency (1). Therefore, this sample can be considered quite representative despite the low numbers in the individual groups. The presence of eosinophils in native airway of pre-symptomatic children provides further evidence about allergic inflammation occurring in the airways quite a long time before development of symptoms and possible clinical diagnosis. This is in keeping with suggestions that airway inflammation builds up in time in those with allergic airway sensitization (21, 28). This highlights the need to identify at-risk children early to prevent ongoing airway inflammation and remodelling.

Acknowledgments
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<th>Mediator pg/ml</th>
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<th>IQR LOCW (n = 9)</th>
<th>Median NW (n = 45)</th>
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LOCW, Late onset childhood wheeze; NW, never wheezed.
Pre-exist airway inflammation in asthma

Department of Health, Personal and Social Services Northern Ireland, Northern Ireland Chest Heart and Stroke Association and Asthma UK. These data have been presented in part at the Annual Meeting of the European Respiratory Society 2009. There is no conflict of interest in this research work.

References