

RELATIONSHIP BETWEEN PAST AIRWAY PATHOLOGY AND CURRENT LUNG FUNCTION IN PRESCHOOL WHEEZERS

Samatha Sonnappa,^{1,2} Cristina M Bastardo,¹ Sejal Saglani,³ Andrew Bush,³ Paul Aurora^{1,2}

1. Portex Unit: Respiratory Medicine and Physiology
UCL Institute of Child Health, London, UK

2. Department of Respiratory Medicine
Great Ormond Street Hospital for Children NHS Trust, London, UK

3. Department of Paediatric Respiratory Medicine
Royal Brompton Hospital and Imperial College, London, UK

Corresponding Author:

Dr. Samatha Sonnappa

Portex Unit: Respiratory Medicine and Physiology

UCL Institute of Child Health and Great Ormond Street Hospital for Children

30, Guilford Street

London WC1N 1EH

Email address: s.sonnappa@ich.ucl.ac.uk

Tel: +44 20 7905 2382

Fax: +44 20 7829 8634

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ABSTRACT:

Introduction: The functional outcome in preschool severe wheezers with eosinophilic airway inflammation and increased reticular basement membrane (RBM) thickness is unknown. We investigated the relationship between airway pathology at age 2y and lung function at age 4-6y in previous severe wheezers.

Methods: Severe wheezers previously investigated with endobronchial biopsy, and healthy children aged 4-6y were recruited. Lung clearance index (LCI), conducting zone ventilation inhomogeneity (S_{cond}), acinar ventilation inhomogeneity by multiple-breath washout; plethysmographic specific airways resistance, and FeNO were measured. Lung function was compared between wheezers and healthy controls, and in wheezers correlated with past RBM thickness and mucosal eosinophilia (EG2⁺ cells).

Results: Seventy-two healthy controls and 28 previous severe wheezers were tested. Wheezers had significantly higher median LCI (6.8 vs. 6.6; $p=0.001$) and S_{cond} (0.046 vs. 0.016; $p<0.0005$) than healthy controls. Past RBM thickness ($r=0.474$, $p=0.047$) and EG2⁺ cells ($r=0.552$, $p=0.041$) showed significant correlations with current FeNO, but no correlations were seen between past RBM thickness and current lung function.

Conclusions: RBM thickness and EG2⁺ cells at age 2y show a significant positive association with FeNO at age 5y. Although lung function was abnormal at age 5y in severe wheezers, this did not correlate with past RBM thickness.

Word Count: 198

Key words: Children; eosinophilia; FeNO; lung function; preschool wheeze; RBM thickness

INTRODUCTION

Asthma comprises different phenotypes that manifest with similar clinical symptoms. Established risk factors for the development of asthma in childhood include frequent wheezing during the first three years of life, a parental history of asthma, personal history of eczema, allergic rhinitis, wheezing apart from colds, peripheral blood eosinophilia of 4% or greater and early allergic sensitization to perennial aeroallergens.[1,2] Some, but not all early wheezers have worsening of lung function, asthma symptoms, or both over time.[3] However, the physiological consequences of pathological changes in the asthmatic airways are poorly understood. While some adult studies show that increased reticular basement membrane (RBM) thickness is associated with airway obstruction,[4] others show that RBM thickening is protective against bronchoconstriction.[5,6] Contemporaneous studies of pathology and function in preschool children with recurrent wheeze are lacking, due to ethical concerns in performing endobronchial biopsies and preschool lung function testing being limited to specialist centres.

We recently reported that preschool children with multiple-trigger wheeze have abnormal lung function whilst those with episodic (viral) wheeze do not, irrespective of atopic and current wheeze status.[7] In a subgroup of these children with severe recurrent wheeze, Saglani et al [8] had previously performed endobronchial biopsies at median age 29 months. They showed evidence of increased reticular basement membrane (RBM) thickness and mucosal eosinophilic inflammation (EG2⁺ cells) in wheezers compared to non-wheezing controls, which was less severe than that seen in adult asthmatics.[8] We hypothesised that in preschool children with previous severe recurrent wheeze, past RBM thickness will show a negative association with current lung function; and mucosal eosinophilic inflammation will show a positive association with an indirect measure of eosinophilic inflammation, namely exhaled nitric oxide (FeNO); and positive association with current wheeze. Therefore, we investigated the relationship between current lung function, FeNO and past endobronchial biopsy findings in this unique cohort; and related these findings to current wheeze phenotype and symptoms.

METHODS

This cross-sectional study was conducted at UCL Institute of Child Health, London, UK and was approved by the Joint UCL/UCLH Ethics Committee. All subjects underwent clinical and lung function assessments, as previously described.[7] Briefly, the assessments included FeNO measured at expiratory flow of 50ml/s using the single breath online method according to the American Thoracic Society guidelines,[9] with computerised equipment and a chemiluminescence analyzer (CLD 88, EcoMedics; Durnten, Switzerland); multiple breath washout indices [lung clearance index (LCI) a measure of overall ventilation inhomogeneity, conducting zone contribution to ventilation inhomogeneity (S_{cond}) and acinar zone contribution to ventilation inhomogeneity (S_{acin})] measured by using sulfur hexafluoride as the inert marker gas;

and plethysmographic specific airways resistance (sR_{aw}). The FeNO measurements reported in this study are after adjusting for the best plateau for a 6-s exhalation.[10] Atopic sensitisation was ascertained by skin prick tests to house-dust mite, cat, dog, grass, tree, and *Aspergillus fumigatus* (Soluprick SQ, ALK-Abello A/S, Horsholm, Denmark); sensitisation was defined as a wheal at least 3 mm greater than the negative control.

The children were re-classified at the study visit in three different ways, according to current wheeze status (Table 1). Subgroup analysis was performed on each of these three-paired categories. FeNO and lung function measurements were correlated with past RBM thickness and EG2⁺ cells (biopsy methods as previously reported) [8,11] in all wheezers and in the wheeze subgroups. By definition, the severe preschool wheezers were between 3 months and 5 years of age and had at least three episodes of wheeze lasting more than 3 days in the previous 6 months.[8] For brevity, previous severe wheezers will be referred to as “wheezers”, irrespective of current wheeze status, throughout the document unless specifying wheeze status according to the current classification in Table 1.

Table 1: Definitions according to wheeze status used in the current study

<p><u>Symptom-pattern status:</u></p> <p>Episodic (viral) wheeze: Wheezing during discrete time periods, often in association with clinical evidence of a viral cold, with absence of wheeze between episodes. [12]</p> <p>Multiple trigger wheeze: Wheezing that shows discrete exacerbations, but also symptoms between episodes triggered by dust, cold air, exercise, laughter, etc. [12]</p>
<p><u>Atopic status:</u></p> <p>Atopic wheeze: Wheezer who is skin prick test positive and/or has current eczema</p> <p>Non-atopic wheeze: Wheezer who is skin prick test negative and has no eczema</p>
<p><u>Current wheeze status:</u></p> <p>Current symptomatic: Wheezer with a history of at least one wheezing episode in the past 12 months</p> <p>Current asymptomatic: Wheezer with no wheezing episodes in the past 12 months</p>

Statistical analysis

Sample size was opportunistic, being determined by the number of children originally enrolled who agreed to return for follow up. Multivariable linear regression was used to compare the differences in pulmonary function between wheezers and healthy controls after adjustment for birth and current weight, age and height. Due to skewness, the outcome variables were log-transformed and adjusted coefficients with 95% confidence intervals are reported. Lung function and FeNO data were log-transformed, to investigate the associations between past pathology findings by the Pearson correlation test. Subgroups of wheezers were compared by using χ^2 or Mann-Whitney U tests, as appropriate. A 5% level of significance was considered statistically significant.

RESULTS

The original ‘severe wheeze’ cohort consisted of 47 wheezers,[8] of whom 28 children (60%) consented to take part in the current study at median age 5.0 (interquartile range (IQR) 4.4 – 5.9) years, 16 males. Seventy-two healthy controls (37 males, median age 5.5 [IQR 5.1-6.2] years) were also recruited. [7] All 28 wheezers were able to perform MBW and sR_{aw} measurements, and FeNO measurement was successful in 22 children. RBM thickness measurements were available for 22 of 28 (79%) children, and data relating to biopsy EG2⁺ cells available for 17 of 28 (61%) children. The median age at which biopsy was performed in the wheezers recruited to the current study was 2.1 years (IQR 1.1 to 3.4 years) and the median interval from endobronchial biopsy to lung function testing was 3.1 years (IQR 2.7 to 3.6 years).

Of the 28 wheezers, 23 (82%) were prescribed inhaled corticosteroids (ICS) in the past. Of these, 15 (65%) children were currently prescribed ICS with equivalent daily doses of beclometasone ranging from 200-800 µg. Eight of these children were prescribed long-acting bronchodilators, four leukotriene receptor antagonists and four antireflux medication (ranitidine or omeprazole and domperidone).

The wheezers had significantly higher LCI, S_{cond} and sR_{aw} compared with healthy controls, but there was no difference in FeNO (Table 2).

Table 2: FeNO and lung function in healthy control subjects and wheezers and multiple linear regression analyses showing the effect of wheeze on outcome measures

Variable	Healthy Controls N (72) Median (IQR)	Wheezers N (28) Median (IQR)	Multiple linear regression analyses		
			Adjusted coefficients* (95% CI)	P value	R ² for model
FeNO (ppb)	4.8 (3.2, 7.6)	4.9 (3.7, 11.4)	1.1 (0.8, 1.7)	0.542	0.036
LCI	6.6 (6.2, 6.9)	6.8 (6.6, 7.5)	1.1 (1.0, 1.2)	0.002	0.204
S _{cond}	0.016 (0.005, 0.026)	0.046 (0.016, 0.065)	3.0 (1.7, 5.2)	<0.0005	0.259
S _{acin}	0.048 (0.029, 0.067)	0.059 (0.033, 0.089)	1.3 (0.9, 1.8)	0.186	0.040
sR _{aw} (kPa.s)	1.03 (0.91, 1.19)	1.18 (0.88, 1.59)	1.2 (1.1, 1.3)	0.002	0.143

**The coefficients are exponentiated and represent the multiplicative effect (95% CIs) of wheeze on the FeNO and lung function outcome. Significant results are indicated in bold.*

IQR – interquartile range

Relationship between past airway pathology and current airway function

There was a significant positive correlation between past RBM thickness and EG2⁺ cells with current FeNO (Table 3, Figure 1). However, past RBM thickness and EG2⁺ cells were not associated with current lung function (Table 3). On exploring these associations further in the current subgroups of episodic (viral) and multiple-trigger; atopic and non-atopic; current symptomatic and asymptomatic wheeze, no significant associations were found between past biopsy findings and current FeNO or lung function.

Past airway pathology and current airway function according to current wheeze phenotype

RBM was significantly thicker and EG2⁺ cells were significantly greater in current multiple-trigger wheezers compared with episodic (viral) wheezers (Table S1 and Figure 2). Current multiple-trigger wheezers also had significantly higher FeNO, LCI and S_{cond}, than current episodic (viral) wheezers despite significantly more multiple-trigger wheezers being currently prescribed ICS (Table S1).

There was no difference in FeNO, lung function and past RBM thickness or EG2⁺ cells between the current atopic vs. non-atopic wheezers (Table S2 and Figure 2). Current asymptomatic wheezers showed a significantly higher S_{acin} than symptomatic wheezers and there was a strong trend for past EG2⁺ cells to be elevated in current symptomatic wheezers compared with asymptomatic wheezers, but there were no other differences (Table S3 and Figure 2).

Table 3: Correlations between current FeNO and lung function with past RBM thickness and EG2⁺ cells

Variable	RBM (mm)	n	P value	EG2 ⁺ cells	n	P value
FeNO	0.474	18	0.047	0.552	14	0.041
sR _{aw}	0.223	22	0.318	0.138	17	0.597
LCI	-0.069	22	0.760	0.127	17	0.627
S _{cond}	0.135	21	0.559	0.009	16	0.974
S _{acin}	-0.106	21	0.647	-0.128	16	0.637

Legend: The reported measure of correlation in the second and fifth columns is the Pearson correlation coefficient. Bold denotes $p < 0.05$; n denotes the number of wheezers with both pathology and outcome data for that variable.

DISCUSSION

This is the first study to investigate the association between past airway inflammation, RBM thickness and lung function, and current symptoms in preschool children with previous severe wheeze. We found that elevation in RBM thickness and EG2⁺ cells at median age 2 years show a significant positive association with FeNO at median age 5 years. Lung function was significantly abnormal in the previous severe wheezers compared to healthy controls, but there was no association between past RBM thickness and current lung function. There was a strong trend for wheezers with elevated EG2⁺ cells at median age 2 years to be symptomatic at median age 5 years.

Saglani et al showed contemporaneously that the RBM was significantly thicker in multiple-trigger wheezers compared with episodic (viral) wheezers[8]. Similarly, another study showed

that children with multiple-trigger wheeze have significantly increased RBM thickness and EG2⁺ cells, irrespective of age and atopic status.[13] It is interesting to note that this difference remains with retrospective rephenotyping even after 3 years, irrespective of presence or absence of symptoms.

There is a positive correlation between FeNO and eosinophilic airway inflammation in adult and paediatric asthmatics when measured contemporaneously,[14,15] and the relationship is better in steroid-naïve patients. However, this is the first study to find an association between directly measured eosinophilic airway inflammation at median age 2 years and FeNO at median age 5 years, in preschool wheezers. Furthermore, the strong trend for current symptomatic wheezers to have had increased EG2⁺ cells compared with asymptomatic wheezers suggests that airway inflammation present at age 2 years can persist three years later, despite prescribed ICS treatment. Nonetheless, this is speculative, as there was no second direct measurement of eosinophilic inflammation, and the relationship is weaker in children using ICS, as in this study. However, we did not think it ethical to stop their prescribed treatment. The relationship may be the result of poor treatment compliance, poor delivery of inhaled medication to the airway, or because ICS are ineffective or only partly effective in modulating inflammation at this age. Our findings give pathological insights into the findings of the PEAK and IFWIN studies,[16,17] in which very early regular administration of ICS failed to modify the progression of asthma. We speculate that children in these studies who went on to wheeze persistently had underlying eosinophilic airway inflammation, which could not be reversed by the prescribed ICS.

The lack of an association between past RBM thickness and wheeze persisting at age 5 years and lung function is in agreement with studies in older children and adults with asthma that were unable to demonstrate a relationship between RBM thickness, duration and severity of asthma [18,19] and lung function.[18,20] Indeed it has been proposed that RBM thickening may be protective rather than deleterious.[5,6] Although our failure to show a relationship between RBM thickness and impaired lung function may be a power issue, it is also compatible with the view that increased RBM thickness does not have adverse physiological effects.[6]

Ethical concerns preclude conducting an invasive longitudinal contemporaneous study investigating the functional consequences of structural changes in children. The sample size and non-contemporaneous nature of our study limits meaningful interpretation of lack of differences and correlations for most of the biopsy findings. A further limitation is that airway smooth muscle thickness was not measured in our biopsies. Studies in older children[21] and adults[22] with severe asthma have shown an association with increased smooth muscle thickness and impairment of lung function. The lung function tests used in this study were comprehensive by including measures of global ventilation inhomogeneity (LCI), conducting zone (S_{cond}) and acinar zone (S_{acin}) contributions to ventilation inhomogeneity and sR_{aw} a measure of airways resistance mainly contributed from the central airways. Nonetheless, the endobronchial biopsies were taken from the sub-segmental carina and it is uncertain whether pathological changes in the central airways reflect changes in the smaller and peripheral airways in young children and whether these in turn are reflected by the physiological measures chosen in this study. However, several studies suggest that airway inflammation occurs throughout the airway,[23,24] and in adults, it has been demonstrated that RBM thickening in the central airways is related to RBM thickening in all cartilaginous airways including the smaller cartilaginous airways, but not the

peripheral membranous airways.[25] There are no such data in preschoolers. The conclusions therefore are more hypotheses generating than definitive. Allowing for these limitations, these are unique data evaluating the relationship between early pathological changes in the airway and later physiological outcomes in preschool children with severe wheeze.

In summary, we have demonstrated that increased RBM thickness and mucosal eosinophilia at median age 2 years shows a significant positive association with FeNO at median age 5 years, in a group of preschool children with severe wheeze. Although lung function was abnormal in these wheezers at median age 5 years, there was no correlation between deficits in lung function and past RBM thickness or eosinophilia. RBM thickness at median age 2 years did not predict presence or absence of wheeze at median age 5 years, but there was a trend for elevated mucosal eosinophilia at median age 2 years to be associated with persistence of wheeze at median age 5 years.

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COMPETING INTERESTS

None.

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Figure 1

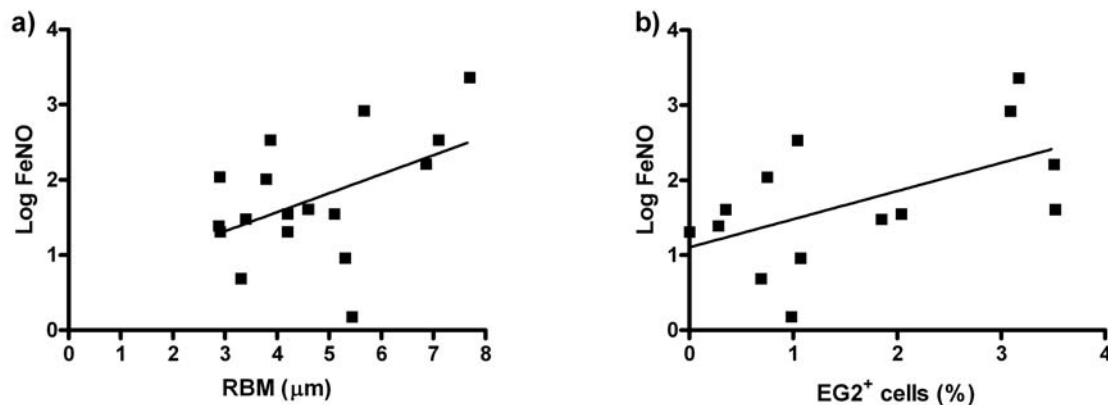


Figure2

