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Comparison of blood eosinophil numbers between acute asthma and stable disease in children with preschool wheeze

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ABSTRACT

Background: Preschool wheezing is common and many children experience exacerbations and are well in between. Raised blood eosinophils in older children are associated with exacerbation-prone wheeze but there are currently no biomarkers to predict near-future exacerbations in preschoolers. There is evidence suggesting eosinophils are acutely activated during exacerbations of preschool wheeze which subsides after recovery using urinary markers however it is unknown whether the profile of leucocytes in the blood differ.

Objective: To investigate whether blood eosinophils numbers differ between an acute wheezing episode and during stable disease in children with preschool wheeze.

Methods: Blood samples for leucocyte differential cell counts were obtained from children aged 10 months to 6 years presenting with acute, doctor-diagnosed wheeze. A repeat blood sample was available in a subset of children after recovery.

Main outcome measures: Difference between blood eosinophil counts during an acute wheezing episode and after recovery (stable disease).

Results: Eighty-five children participated in this study, 68 with acute wheeze (median blood eosinophil count was 0.10 $x10^9$/L (range 0.00-2.41)) and 17 healthy controls. After recovery, blood eosinophils available for 20 children were significantly higher (median 0.43 $x10^9$/L (range 0.12-1.25)). There was no significant difference in blood eosinophil counts between children with preschool wheeze and healthy controls when measured acutely whereas eosinophil counts were significantly higher in children with stable preschool wheeze compared to controls. Blood neutrophil counts fell between the acute episode and after recovery whereas blood lymphocyte counts rose similar to eosinophil counts.

Conclusions: Blood eosinophil numbers are greater during stable disease compared to the exacerbation state. This is an important consideration when planning future studies examining blood eosinophils in relation to preschool wheeze.

Words: 273

KEYWORDS:

Asthma

Pediatric

Blood eosinophils

ABBREVIATIONS:

BTS – British Thoracic Society

GINA – Global Initiative for Asthma

ICS – Inhaled corticosteroids

PPI – Patient and public involvement

SIGN – Scottish Intercollegiate Guideline Network

TBE – Total blood eosinophils

TBL – Total blood lymphocytes

TBN – Total blood neutrophils

INTRODUCTION

Wheezing in the first six years of life is extremely common and affects up to half of all preschoolers by their fifth birthday[1,2]. Severe exacerbations requiring emergency treatment are common in this age group, result in significant stress, have economic implications for caregivers and families and place a considerable financial burden on health services[3].

One important clinical difficulty when dealing with wheezy preschoolers is how to identify children with exacerbation-prone wheeze for a trial of preventer medication. Inhaled corticosteroids are the first line preventer treatment recommended by the BTS/SIGN and GINA guidelines[4,5]. However it is estimated that only approximately half of young children respond to this treatment[6,7]. In addition there are concerns regarding potential side effects of this treatment specifically the reduction in growth velocity[8]. This results in reluctant prescribing and adherence to this medication.

A major clinical advance would be the identification and validation of a widely available biomarker to identify children at high risk of recurrent severe exacerbations. To date no such biomarkers have been described but blood eosinophils have been shown to predict exacerbation frequency in school-age children and adults with asthma[9-11] and eosinophilic asthma is predictive of corticosteroid responsive disease[7]. Elevated blood eosinophils[12,13] have been shown to be risk factors for persistence of preschool wheeze into older childhood. Thus, blood eosinophils are a promising candidate to be studied as a potential biomarker of corticosteroid responsive disease in preschool children. The uncertainty that remains however is whether blood eosinophil counts change depending on whether the preschool child is acutely unwell with wheezing or whether they are in a period of stable disease between

exacerbations. This is an important issue to address as this could affect how samples are taken in such studies.

Therefore, our principal research aim was to investigate whether blood eosinophil counts (and neutrophil and lymphocyte counts) differed between an acute wheezing episode and after recovery in preschool children.

METHODS

Participants

We conducted a prospective observational study. Between October 2014 and May 2015 children aged between 10 months and 6 years treated in the children's emergency department at University Hospitals Leicester, UK, for an exacerbation of doctor-diagnosed wheeze were eligible to participate if they had at least one previous parent-reported episode of wheezing. Children with a presumed clinical diagnosis of bronchiolitis or those with complex medical problems including moderate to severe prematurity were excluded. Children who had received oral corticosteroids for more than 24 hours prior to potential recruitment were not approached due to the systemic corticosteroid effect on blood eosinophil numbers[14].

Parents were approached at the earliest opportunity after registering their child in the emergency department. Information was gathered regarding the child's wheezing history and currently prescribed asthma medications. In children where a clinically directed blood test was performed an additional sample of blood was obtained for a leucocyte differential cell

count. In the remaining children either a blood sample was drawn by venepuncture or finger prick, depending on parental preference.

Parents were asked for permission to approach them again for a repeat sample once their child had completely recovered from the acute wheezing episode and no earlier than four weeks following discharge home from hospital. All blood samples were taken in accordance with the infection control policies at our hospital and the assistance of a play specialist was sought to minimise distress to the children wherever possible. Data on subsequent wheezing episodes and unscheduled healthcare visits due to wheeze was also collected either at the follow-up visits in hospital or via the telephone at times and dates agreed with the parents.

Control participants who had no previously documented or parent-reported wheezing episodes or significant comorbidities were recruited from pre-operative assessment clinics prior to routine ear, nose and throat procedures. A sample of blood was taken in theatre during induction of anaesthesia from the indwelling intravenous cannula placed by the anaesthetist. The Research Ethics Committee (Nottingham, UK) approved all aspects of this study (NRES reference 09/H043/92). Parents or legal guardians provided informed, written consent. The study design is summarised in figure 1.

(Figure 1: Study design)

Laboratory testing

Blood samples were processed in our haematology laboratory or by a near patient testing full blood count analyser (Sysmex xs800i: Sysmex Europe GmbH, Norderstedt, Germany) in our emergency department. This equipment uses fluorescent flow cytometry to determine a leucocyte differential[15]. The device is checked, calibrated and quality controlled daily by our haematology laboratory personnel.

Statistical analyses

The analyses were performed using Statistical Package for Social Sciences (SPSS, version 22) and Graphpad Prism (version 6.04). Total blood eosinophil, neutrophil and lymphocyte counts were not normally distributed hence non-parametric statistical tests were used. Mann-Whitney U-test was used to assess for statistically significant differences between total blood eosinophil counts in children with preschool wheeze and controls. Wilcoxon signed rank test was used to assess differences between inflammatory leucocytes between the acute episode and after recovery. Significance was assessed at the 0.05 level.

RESULTS

Participants

During the study period, 181 parents of children fulfilling the inclusion criteria for acute preschool wheeze were approached, of which 74 agreed to participate and an adequate blood sample was obtained from 68 children, 36 by venepuncture and 32 by finger prick sampling. We found no significant differences in total blood eosinophil counts or total blood neutrophil counts between children with acute wheeze who had or who had not received systemic corticosteroids prior to their blood sample being drawn (data not shown). The consort recruitment diagram is shown (Figure 2). Seventeen control children were also recruited during this time period. The demographic and clinical characteristics of the study children are comparable between the two groups (Table 1). Twenty families agreed for a repeat sample to be taken after the child had recovered from the acute wheezing episode.

(Figure 2: Recruitment algorithm)

Table 1: Demographic and clinical characteristics of children

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Comparison of inflammatory cell numbers between acute and stable disease

Paired samples were available for 20 children. During stable disease, total blood eosinophils and total blood lymphocyte were significantly higher compared to during the acute exacerbation. In contrast, total blood neutrophil counts fell significantly after recovery (Table 2, Figure 3).

Table 2: Blood leucocyte differential cell counts in paired, acute and stable samples

(Figure 3: Absolute blood leucocyte counts during acute wheeze and following recovery for 20 children)

Blood eosinophil counts and near-future exacerbations

Follow-up data on subsequent exacerbations was available for 35 children which included the 20 who returned for a repeat blood sample. Follow-up data was not available in 33 children (4 had declined further contact at recruitment and 29 were not contactable for follow-up). The median follow-up time for these 35 children was 13 weeks (range 7-30weeks). There was no association between blood eosinophil numbers measured during the exacerbation and near-future exacerbations. We found that stable eosinophil counts were significantly higher in those children who had subsequent exacerbations of wheezing in comparison to those who had not. However due to the paired sample size being relatively small there is insufficient power to draw reliable conclusions on this aspect of the study.

DISCUSSION

The key finding of our study is that leucocyte counts significantly differ between an acute episode of wheezing and stable disease in children with preschool wheeze. We found that eosinophil counts were significantly higher after recovery of the acute episode, as were lymphocyte counts. Blood neutrophil counts fell between acute wheezing and after recovery. Moreover, whilst there was no significant difference between blood eosinophil counts between those children with preschool wheeze and control children, those with stable preschool wheeze had significantly higher blood eosinophil counts.

The biomarker 'blood eosinophils' has emerged as a potentially attractive candidate in preschool wheeze given its use as a marker of disease activity in asthma in older children and adults. Three large recent epidemiological studies in children aged six years and above and adults with asthma have shown that blood eosinophils are associated with exacerbation-prone asthma[9-11]. Malinovschi *et al* reviewing the laboratory markers of more than 12,000 individuals with asthma aged 6-80 years found that peripheral blood eosinophils of more than 3% are independently associated with emergency healthcare visits due to exacerbations[10]. This finding has been confirmed by a separate study reviewing data from 3,162 subjects with asthma from the National Health and Nutrition Examination Survey, an annual cross-sectional survey of the US general population, where the authors found that the presence of absolute blood eosinophil counts $\geq 0.3 \times 10^9$/L was associated with an increased frequency of acute asthma attacks in respondents, particularly in children[9]. There is to date no data in young children with preschool wheeze.

To our knowledge this is the first study to systematically investigate whether blood eosinophils, and indeed other leucocytes, differ between acute wheeze and stable disease in children with preschool wheeze. We are not aware of previous reports in children comparing acute and stable blood eosinophil numbers in children with preschool wheeze or asthma. Using urinary markers however, one previous study reported that eosinophil activation subsided after recovery from an acute wheezy exacerbation[16]. Blood eosinophil counts were measured in the acute episode in these children however were unfortunately not measured after recovery. An additional study conducted in infants with bronchiolitis reported a rise in total blood eosinophil counts after recovery from the acute episode[17].

We are aware that circulating blood eosinophils follow a circadian rhythm with higher levels after midnight and in the early morning hours[18]. None of the children recruited in our study had blood eosinophils taken during the night and blood eosinophil counts in the majority of children studied were obtained between 9am and 9pm. The reasons for the greater number of blood eosinophils during stable periods are not entirely clear but it is possible that blood eosinophils are recruited into the lung during acute exacerbations[7] potentially leading to a lowering of absolute blood eosinophil counts at this stage. This theory is certainly supported by the evidence demonstrating eosinophil activation during acute wheezing in preschool children which subsides after recovery from the episode[16]. However, we were not able to investigate airway eosinophils in parallel to blood eosinophils. It would be difficult to justify performing a bronchoscopy and bronchoalveolar lavage in preschoolers with an acute wheezy exacerbation and indeed after recovery.

Study strengths and limitations

Performing studies in young children that involve blood sampling are challenging by their very nature. Despite this limitation a relatively large number of children with acute preschool wheeze was recruited. Obtaining follow-up blood samples when the children were clinically well was more difficult. It is perhaps not surprising that parents were reluctant to allow their children to have a blood test at a time when they were well. Despite this we obtained a repeat blood sample in 20 children which allowed us to make a meaningful comparison between acute and stable blood leucocyte differentials. During a patient and public involvement initiative prior to study initiation, approximately half the participating parents expressed a preference for a finger prick blood sample rather than venepuncture and this choice was incorporated into the ethics protocol. Furthermore, our data is strengthened by the relatively large control group recruited as part of this study.

Given the lack of an identified and validated profile of preschool children likely to respond to corticosteroid treatment it is important that potential biomarkers such as blood eosinophil counts are investigated. Within this context, this study adds an important finding that blood eosinophil count numbers change between an acute episode of wheezing and when stable and it is imperative that this is taken into consideration in such studies in the future.

Conclusions

We investigated whether there were differences between leucocyte counts in children with acute preschool wheeze and during stable disease. The finding that blood eosinophil counts were significantly higher during stable disease in comparison to acute wheeze is an important consideration for future studies investigating this biomarker in relation to preschool wheeze.

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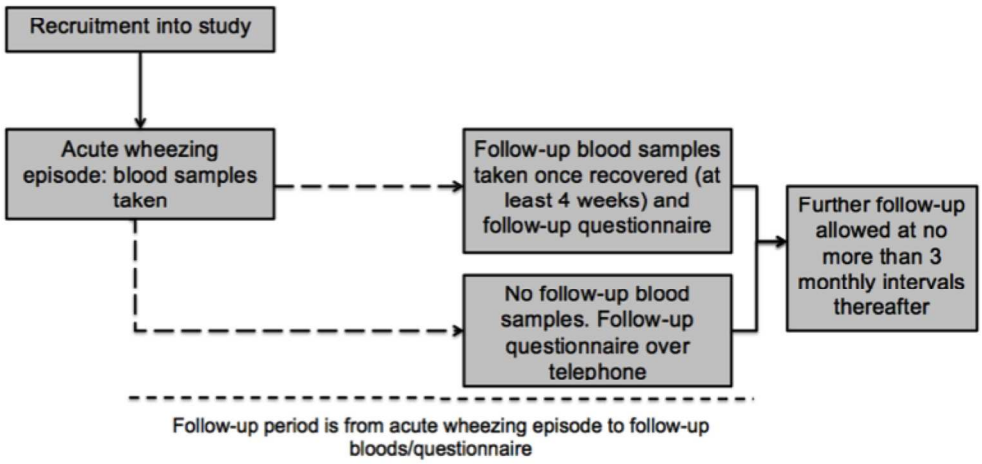

Study design

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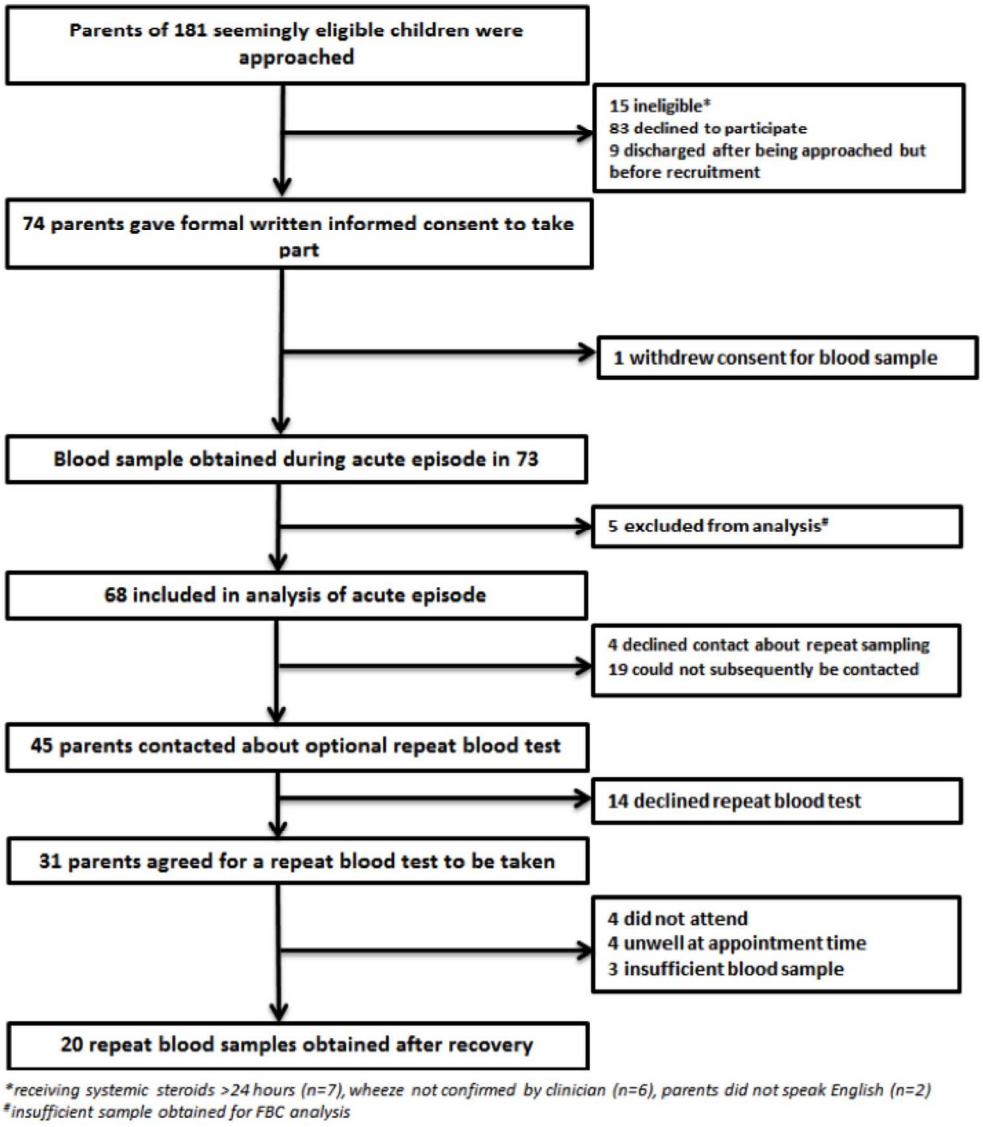
Figure 2: Recruitment algorithm

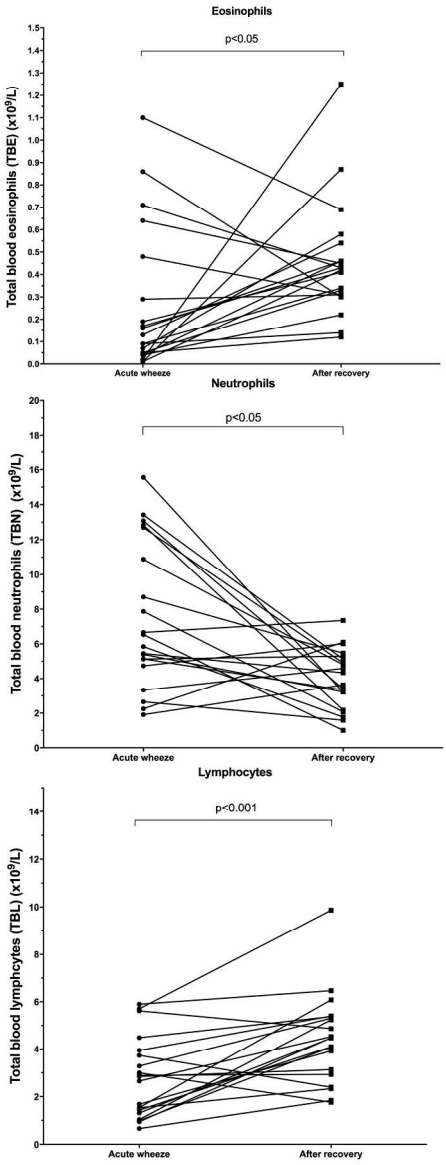


*receiving systemic steroids >24 hours (n=7), wheeze not confirmed by clinician (n=6), parents did not speak English (n=2)
#insufficient sample obtained for FBC analysis

Recruitment algorithm

167x201mm (300 x 300 DPI)

Figure 3: Absolute blood leucocyte counts during acute wheeze and following recovery for 20 children



Absolute blood leucocyte counts during acute wheeze and following recovery for 20 children

191x265mm (300 x 300 DPI)

Table 1: Demographic and clinical characteristics of children

	N	Preschool wheeze (n=68)	N	Control children (n=17)	p value
Sex					
Male, *n* (%)		42 (61.8)		14 (82.4)	0.10
Age (months)		31.2 (10.7 – 70.3)		39.5 (11.6 – 70.4)	0.212
Ethnicity					
White, *n* (%)		48 (70.6)		17 (100)	0.08
South Asian, *n* (%)		15(22.1)		0 (0)	
Mixed, *n* (%)		3 (4.4)		0 (0)	
Other non-white, *n* (%)		2 (2.9)		0 (0)	
History of atopy[#], *n* (%)		33 (48.5)		5 (29.4)	0.156
Positive parental history of wheezing or asthma, *n* (%)		29 (42.6)		4 (23.5)	0.148

BTS step					
Step 0, *n* (%)		25 (36.8)		N/A	N/A
Step 1, *n* (%)		26 (38.2)			
Step 2, *n* (%)		16 (23.5)			
Step 3, *n* (%)		1 (1.5)			
Total blood eosinophil (TBE) count					
Acute wheeze TBE ($x10^9$/L)	68	0.10 (0.00-2.41)	0	N/A	N/A
Stable disease TBE ($x10^9$/L)	20	0.43 (0.12-1.25)	17	0.17 (0.00-0.83)	<0.05
Total blood neutrophil (TBN) count					
Acute wheeze TBN ($x10^9$/L)	68	7.07 (1.91-27)	0	N/A	N/A
Stable disease TBN ($x10^9$/L)	20	3.99 (1-7.35)	17	4.77 (2.24-15.0)	<0.05
Total blood lymphocyte (TBL) count					

Acute wheeze TBL ($x10^9$/L)	68	2.62 (0.66-12.64)	0	N/A	N/A
Stable disease TBL ($x10^9$/L)	20	4.48 (1.76-9.87)	17	4.39 (2.16-7.62)	<0.05

N= number of samples available [#]Atopic status determined by parental report of doctor-diagnosed eczema or allergic rhinitis. All data is presented as median (range) unless otherwise specified.

Table 2: Blood leucocyte differential cell counts in paired, acute and stable samples

	Acute wheeze (n=20)	After recovery (n=20)
TBE ($x10^9$/L)	0.11 (0.01-1.10)	0.43 (0.12-1.25)*
TBN ($x10^9$/L)	6.19 (1.91-15.6)	3.99 (1.0-7.35)*
TBL ($x10^9$/L)	2.74 (0.66-5.90)	4.47 (1.76-9.87)*

* stable blood leucocytes significantly different ($p<0.05$ for eosinophils and neutrophils; $p<0.001$ for lymphocytes) compared to acute paired sample.

TBE: Total blood eosinophils; TBN: Total blood neutrophils; TBL: Total blood lymphocytes.

Data displayed as median (range).