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Eosinophil cell count in bronchoalveolar lavage fluid in early childhood wheezing: is it predictive of future asthma?

Guy Gut, MD^a, Keren Armoni Domany, MD^b, Efraim Sadot, MD^b, Ruth Soferman, MD^b, Elizabeth Fireman, PhD^c, and Yakov Sivan, MD^d

^aPediatric Pulmonary Institute, Ruth Children's Hospital, Rambam Health Care Campus, Haifa, Israel; ^bThe Department of Pediatric Pulmonology, Critical Care and Sleep Medicine, "Dana-Dwek" Children's Hospital, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; ^cInstitute for Pulmonary and Allergic Diseases, and National Laboratory Service for Interstitial Lung Diseases, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; ^dDepartment of Pediatric Pulmonology, Safra Children's Hospital, Sheba Medical Center, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel

ABSTRACT

Objective: Increased eosinophil level in bronchoalveolar lavage fluid (BALF) characterizes asthma in school-age children and adults and has been suggested as a marker for disease severity and response to treatment. We aimed to investigate the occurrence and yield of BALF eosinophil cell count in preschool children with recurrent wheezing and its possible relation to future diagnosis of asthma. **Methods:** BALF was retrospectively studied in young wheezy children and its relation to asthma at age 6 years was evaluated. BALF from children aged 1–48 months (mean = 20.4) was analyzed in preschool wheezy children. Children with anatomical airway obstruction and other lower airway/lung diseases who underwent BALF served as controls. Assessment of asthma was accomplished at 6 years. **Results:** Eighty-two children were included. The mean age during bronchoscopy and BAL was 20.4 ± 14.4 months (range: 1–48 months). Twenty-six patients had recurrent preschool wheezing, 13 anatomical airway obstruction and 43 had other lower airways/lung diseases. Groups were comparable for age during bronchoscopy and gender. No difference was found between groups for any of the BALF cell types. Eosinophils were very low in all three groups [mean (interquartile range): 0 (0–0.4), 0 (0–0.8), and 0.4 (0–1), respectively, $p = 0.25$]. No difference in eosinophil levels during bronchoscopy was found between asthmatic children to non-asthmatic as defined at age 6 years. **Conclusions:** Wheezing in preschool children is not associated with increased BALF eosinophils; hence, at this age, the diagnostic yield of BALF for cell count analysis for diagnosing asthma is limited and is not routinely indicated.

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Introduction

Flexible bronchoscopy with analysis of bronchoalveolar lavage fluid (BALF) is an essential part in the evaluation of a variety of lung diseases in children, including severe and unresponsive wheezing. Guidelines and reference values for cellular and non-cellular components of BALF have been reported [1–3]. Asthma in children is commonly an allergic disease showing increased eosinophil level in BALF of school-age children and adults [4–10]. BALF and peripheral eosinophil level has been suggested as a marker for asthma, disease severity and response to systemic and inhaled corticosteroids treatment [11–16].

Preschool wheezing is very common and may affect more than one third of children under the age of 3 years [17,18]. Most of these children have transient

conditions outgrowing their airway disease before they reach school age and do not exhibit any future wheezing. However, in a minority preschool wheezing is associated with future asthma [17].

Diagnosis of asthma relies upon a typical clinical presentation combined with demonstration of reversible airway obstruction and response to controller medications [19]. Some children require further evaluation that includes laboratory confirmation of reversible airway obstruction, challenge tests and allergy workup (skin prick-tests and/or IgE). In selected patients with severe persistent wheezing, flexible bronchoscopy with bronchoalveolar lavage (BAL) is also performed in the diagnostic process, mainly to exclude other causes for airway obstruction, to search for infection and for the analysis of BALF [20,21].

Unfortunately, lung function testing is very difficult to perform in preschool children especially younger than 4 years. This makes the diagnosis of asthma in preschool children very challenging. In addition, the nature and pathogenesis of asthma in 1–4 years old children may differ from school-age asthma in that it is mostly triggered by viral respiratory infections rather than by allergens and a large portion of patients do not have an atopic background. Hence, the yield of allergic workup is limited [22]. Whether analysis of BALF as a relatively objective tool provides an added value in the diagnosis of asthma or a tendency for future asthma in small children is unclear especially since bronchoscopy and BALF analysis are indicated only in selected cases.

Data evaluating eosinophil levels in the BALF of wheezy infants and toddlers is scarce [16,23–26]. While most of these reports found low eosinophil levels, due to ethical issues, comparison to a control group was available in only few studies and more important, data regarding association with future occurrence of asthma was not included. Thus, the question whether eosinophil levels in the BALF can add to predict asthma in symptomatic young children is still open.

The aim of this study was to investigate whether BALF in preschool children with recurrent wheezing who underwent bronchoscopy exhibits alterations in differential cell count and specifically in eosinophils count and to evaluate the yield of the findings to the future occurrence of asthma.

Materials and methods

In this retrospective study, we retrieved the data on consecutive children younger than 4 years who underwent flexible bronchoscopy and BAL between July 2014 and July 2016. In our institution, cell differential is done routinely on every BALF sample. Excluded were children with hemato-oncologic disorders or immune deficiency because of probable effect of their underlying disease or treatment on BALF cellular constituent. All bronchoscopies were performed for clinical indications. Indications for bronchoscopy are reported in Table 1. None of the patients received systemic corticosteroids within the month preceding the procedure or inhaled corticosteroids within 96 h prior to the procedure.

Bronchoscopy was performed under sedation with intravenous midazolam, ketamine and propofol and local anesthesia to the larynx and trachea with lidocaine and continuous monitoring of ECG, breathing,

Table 1. Baseline age, gender, and indication for bronchoscopy.

Group	Preschool wheezing	AW obstruction	Lower airway and lung disease	P
Mean age (months)	24	17	17	0.12
Gender (% males)	50	85	56	0.15
Indication				
Expiratory noise [†]	10 (38) [‡]	7 (53)	7 (16)	
Inspiratory noise	–	4 (31)	–	
Chronic cough	5 (19)	–	3 (7)	
Radiologic abnormalities	8 (30)	–	22 (51)	
Other	3 (11)	2 (16)	11 (26)	
Total	26	13	43	

[†]Recurrent or unremitting wheezing with poor or no response to treatment.

[‡]In bracket percent from the total group.

oxygenation and blood pressure. Oxygen supplementation via nasal insufflation of 2–3 L/min. was added. All procedures were video-recorded.

Bronchoscopy started with exploration of the tracheo-bronchial tree for anatomical and functional abnormalities after which the bronchoscope was wedged and BAL was performed. No suction was applied above the glottis opening. The site of BAL was determined according to clinical, radiological or endoscopic findings. BAL fluid recovery and processing was made according to standard protocols and guidelines into specimen traps [3]. The first aliquot was not used for analysis since it reflects mainly bronchial milieu rather than pure alveolar material and cells [3]. The collected specimens were filtered through sterile gauze and centrifuged at 400 x g for 15 min at 4°C. The pellet was washed three times with cold phosphate-buffered saline (PBS; Biological Industries, Beit Haemek, Israel) and the cells were adjusted to a final concentration of 10⁶ cells per milliliter in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2% fetal calf serum (Biological Industries). One hundred microliters of this suspension were loaded into cuvettes of a cyto-centrifuge (Cytospin; Shandon, Southern Products Ltd, Runcorn, Cheshire, UK). The slides were spun at 1,000 rpm for 5 min and the fluid blotters were removed. After the slides were dried in air, a differential count was performed on Giemsa (Merck, Darmstadt, Germany) stained preparations by counting a minimum of 500 cells. Cell differential results were presented as percent of total count. All BALF specimens were sent also to microbiology studies including bacterial, tuberculosis and fungal cultures. A positive bacterial culture was defined as growth of at least 10⁴ CFU/ml [1,2].

All children were followed in the Pediatric Pulmonology Unit. Final diagnosis was concluded by a certified pediatric pulmonologist who was following

the patient and was based on medical history, physical examinations on repeated visits, laboratory tests, imaging studies, bronchoscopy results and response to bronchodilators and controller medications when indicated.

In order to investigate the characteristics of BALF cells and the yield of BALF cell differential count to diagnosis in preschool wheezy children, BALF analysis in these children were compared to two control groups according to their final diagnosis:

1. Preschool wheezing: reversible obstructive airway disease could be determined clinically, wheezing was documented at least in two episodes, patient responded to controller medications and other diagnoses were ruled out (cystic fibrosis, anatomical obstruction etc.). In addition, diagnosis of asthma was re-assessed by the pediatric pulmonologist when the child reached 6 years of age using the “International Study of Asthma and Allergies in Childhood” (ISAAC) core questionnaire [27].
2. Control group 1 – anatomical airway obstruction above the carina: children with: laryngomalacia, hemangioma, vascular compression, tracheomalacia, etc., without any lower airway or lung disease.
3. Control group 2 – non-asthma lower airways or lung disease: patients with: bronchiolitis obliterans, non-CF bronchiectasis, aspiration pneumonia etc.

The study was approved by the local and Ministry of Health IRB (Helsinki Committee).

Statistical analysis

Cell differential were expressed as a percentage of total cell counts. Continuous variables are presented using mean and SD (for normally distributed results) and median and interquartile ranges for non-normally distributed and nonparametric data. Categorical variables are presented using percentages. Continuous variables were compared between groups using the Mann-Whitney test or the Kruskal-Wallis test. Comparisons of variables according to group assignment were performed using independent t-tests or analysis of variance, followed by post-hoc comparisons, with *p*-values adjusted for unequal variances when appropriate (Levene’s test for equality of variances), or χ^2 analyses with Fisher’s exact test (dichotomous outcomes). Correlations were performed using

linear regression, followed by calculation of Pearson correlation coefficients. All *p*-values reported are two tailed, with statistical significance set at less than 0.05. All analyses were carried out using SPSS software (version 21; SPP Inc., Chicago, IL, USA) by the Laboratory of Statistics of the Tel Aviv University.

Results

Eighty-two children were included. The mean age at the bronchoscopy and BAL was 20.4 ± 14.4 months (range: 1–48 months). The diagnosis in twenty-six children was preschool wheezing, 13 had anatomical airway obstruction and 43 suffered from a variety of other lung diseases (Table 1). Groups were comparable for age during bronchoscopy and gender (Table 1). Indications for bronchoscopy and BAL are presented in Table 2. Overall, patients were referred for bronchoscopy and BAL because they either responded poorly to treatment, to rule out anatomical airway obstruction, a foreign body, or because they had a right middle lobe syndrome. Analysis of the wheezy children group at age 6 years showed that out of the 26 patients, 12 still had asthma by ISAAC criteria [27], in 11 children asthma was ruled out and 3 were lost to follow up.

BALF results

Differential cell counts from BALF are presented in Table 3. There was no difference in the percentage of any of the cell types between the three groups. Eosinophil levels were very low in almost all patients with no difference between groups (Table 3 and Figure 1). 18/26 children with preschool wheezing did not have any eosinophils in their BALF. Only one patient (7%) in the anatomic airway obstruction group

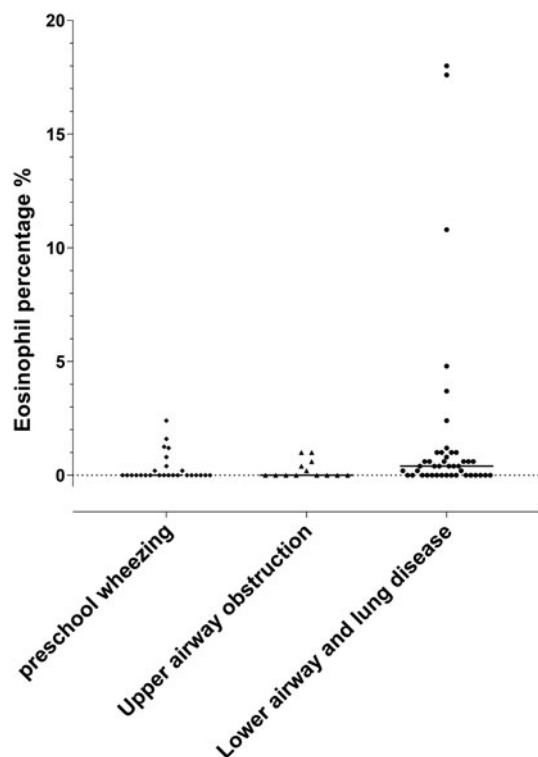
Table 2. Diagnosis after bronchoscopy.

Groups (n)
Preschool wheezing (26)
Upper airway obstruction (13)
Vascular compression (7)
Laryngomalacia (2)
Pharyngomalacia (2)
Rule out of foreign body aspiration (2)
Lower airway and lung disease (43)
Bronchiolitis obliterans (9)
Recurrent or non-resolving pneumonia (7)
Non-CF bronchiectasis (5)
Recurrent aspiration (5)
Bronchomalacia (4)
Atelectasis (3)
NEHI (2)
CPAM (2)
Other (6)

Table 3. Medians and interquartile ranges of differential cell counts from BALF according to group.

Group	Preschool wheezing median (IQR)	AW obstruction median (IQR)	Lower airway and lung disease median (IQR)	P
N	26	13	43	
PMN %	26 (7–56)	38 (4.5–74)	40 (9–71)	0.82
LYMP %	19 (11–29)	11 (8–20)	16 (8–30)	0.47
EOS %	0 (0–0.4)	0 (0–0.8)	0.4 (0–1)	0.25
MAC %	48 (22–69)	37 (15–83)	25 (11–58)	0.3

IQR: interquartile range.

**Figure 1.** BALF eosinophil percentage by diagnosis.

and 5 (11.6%) with lower airways and lung disease had relatively high eosinophil levels (3.7–18%).

Sub-group analysis of children with preschool wheezing according to patient's status at the age 6 years or later, showed no difference in eosinophil levels between the 12 patients who still had symptoms of asthma compared to the 11 children who had recovered with mean eosinophil levels of 0 for both sub-groups: asthmatic children (0–1.2), non-asthmatics (0–0) (interquartile range), $p = 0.12$.

There was high variability in polymorphonuclear cells (PMNs) rate among children in each of the three groups but no difference between groups. Other cell types also did not differ between groups (Table 3).

Positive bacterial cultures ($> 10^4$ CFU) were found in 30% of the cases with *hemophilus influenza* being

the most common. The rate of a positive BALF culture was similar between groups (41%, 31% and 21% for the preschool wheezing, anatomical obstruction and lower airways and lung disease groups, respectively, $p = 0.23$). The only variable that correlated with positive bacterial cultures was PMNs percentage in the BAL ($p = 0.001$).

Discussion

This study shows that eosinophil levels in BALF of wheezy asthmatic infants and children younger than 4 years of age are not elevated compared to non-wheezy infants and toddlers and that the levels are overall very low. This was true also for young children who remain symptomatic for asthma at age 6 years. Indeed, their eosinophil levels were within the reported range for normal healthy children, i.e., < 1.0 – 1.2% [28–30]. This suggests that the eosinophilic inflammation commonly found in the sputum of older children with asthma is absent at a younger age and develops only later. Using tissue studies obtained from endobronchial biopsies, Saglani et al. have shown that the characteristic pathologic features of asthma in adults and school-aged children including tissue eosinophilia develop in preschool children with confirmed wheezing between the ages of 1 and 3 years [31,32]. These findings correlate with ours, although the relationship of bronchial to BAL eosinophilia is not always clear [33]. In accordance with the lower occurrence of other allergic manifestations in young asthmatic or wheezy children, such as positive skin prick-tests and increased IgE levels [34], the present findings provide an additional support for the concept that eosinophilic inflammation, as far as can be delineated by BAL analysis, does not play a major role in younger wheezy children (< 4 years old) compared to older preschool and school-age asthmatic children. This also coincides with the inconsistent response to inhaled corticosteroids as controllers for asthma in lower ages [35].

Similar to our findings, Le Bourgeois et al. found very low levels of eosinophils in BALF of children with severe recurrent wheezing younger than 3 years [23]. Similar results were reported by Marguet et al. but their control group was older (1.5–15 years old) [24]. Other studies found that unlike older asthmatic children whose BALF showed increased eosinophil levels, only very low levels of eosinophils were observed in wheezy infants and toddlers [16,25,26]. These latter studies did not include a control group.

Conflicting findings were reported by Thavagnanam et al. who showed that increased airway eosinophils in asymptomatic non-asthmatic healthy children was associated with the development of asthma later in life [36]. However, this study looked at late-onset asthma and used a blind non-bronchoscopic sampling and did not discard the first sample.

In addition, these previous studies did not analyze the results according to the presence of asthma at school age. We found that children who will suffer from asthma later also had low eosinophil levels in their airways at young age. It is reasonable to assume that children with asthma at age 6 years who started to wheeze as infants or toddlers and in whom asthmatic symptoms have not resolved, had their asthma start at young age and that their disease is a continuum and not two different diseases at young and at school ages. If so, our findings support the theory that even in asthmatic children who started their disease early, allergic mechanisms may become apparent only later.

Unlike previous studies [23,24], PMN count was not increased in our cohort, but was related to bacterial growth. The association between PMN levels and positive bacterial culture in our patients supports the validity of our BALF samples.

Our study has several limitations. The definition of asthma at young age relying only on clinical presentation and response to therapy may be difficult. This is indeed the accepted method in this age group both in studies and in real life [5]. We, therefore, preferred to use the term preschool wheezing. We limited inaccuracies in the diagnosis by not including patients solely on "physician diagnosed asthma" as applied in many other studies, but insisted that the diagnosis would be confirmed by certified and experienced pediatric pulmonologists who personally followed each of the cases until the diagnosis could be determined.

The present study included only children who underwent bronchoscopy and BAL for clinical indications (Table 2). Hence, this was not a cross sectional study of consecutive or randomized cohorts of wheezy infants and toddlers implying a selection bias by excluding those patients who did not meet clinical indications for the procedure, hence those who were studied had a more severe disease. We believe that it is not ethical to suggest the procedure also in infants and small children who do not require it for clinical considerations. Nevertheless, since any positive findings are expected to manifest mainly in the more severe or less controlled patients and only the more severe cases and less controlled were included, it may

be reasonable to assume that if changes in BALF cellular profile existed, they should have appeared in the more severe patients. Hence, the bias was toward a higher chance of finding BALF eosinophilia in the studied cases since no increased eosinophils was observed in the sicker wheezing/asthmatic young children, the findings hold also for those who did not meet the indications for the procedure.

Theoretically, it is possible that BALF eosinophils may only present in preschool wheezers when they actually wheeze and not during the non-symptomatic intervals. Nevertheless, increased eosinophil count does occur in school-age asthmatics also between episodes [6,10–13]. Moreover, it has been recently shown that increased blood eosinophil number is more likely to be found during stable disease rather than during acute episodes [37]. Even if this situation occurs in young wheezers, it still supports the findings that the yield of BALF analysis in the process of evaluation for asthma diagnosis in preschool wheezers is low.

We did not adjust the results for family history including allergy or smoking. Nevertheless, the fact that the overall eosinophil levels were low implies that these would not have affected the results.

In conclusion, unlike school-aged children, asthma symptoms in young children are not associated with increased BALF eosinophil levels. Hence, the yield of bronchoscopy for BALF analysis and eosinophil count for diagnosing asthma is very low under 4 years of age and should, therefore, be performed only when other indications co-exist. The findings also suggest that the eosinophilic proliferation in the bronchoalveolar fluid that characterizes asthma develops later in life and is not apparent during the first years. These conclusions hold also in children who have a later school-age established asthma.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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