

Bronchoalveolar lavage in asthmatic children: Evidence of neutrophil activation in mild-to-moderate persistent asthma

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Little information is available on cell profiles and mediator production in the lower airways of children with asthma by comparison with the adult population. To study the bronchoalveolar lavage (BAL) cell profiles and production of eosinophil cationic protein (ECP) and myeloperoxidase (MPO) in childhood bronchial asthma, a retrospective study was performed in 29 children (13 allergic asthmatic children and 16 controls). Six of the asthmatics had mild-to-moderate persistent disease and seven had intermittent asthma. The BAL cell count and ECP and MPO values of asthmatic children were compared with those from 16 controls. The asthmatic patients had higher values than controls for the total cell count ($p=0.08$), for neutrophils ($p=0.02$), and for ECP and MPO ($p<0.001$). MPO levels ($p=0.04$), neutrophil count ($p=0.06$), and ECP values ($p=0.06$) were higher in patients with mild-to-moderate persistent asthma than in those with intermittent asthma. Our results demonstrate that neutrophil-mediated inflammation is greater in patients with more severe asthma.

**Angelo Barbato¹, Cristina Panizzolo¹,
Marta Gheno¹, Laura Sainati¹,
Elisabetta Favero², Diego Faggian³,
Franca Giusti⁴,
Lidia Pesscolderung⁵ and
Mario La Rosa⁶**

¹Department of Pediatrics, ²School of Allergy,

³Department of Laboratory Medicine and

⁴Department of Anesthesiology, University of Padova, Padova, Italy, ⁵Pediatric Division, Bolzano Hospital, Bolzano, Italy, ⁶Department of Pediatrics, University of Catania, Catania, Italy

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Prof. Angelo Barbato, Department of Pediatrics, Via Giustiniani 3, 35128 Padova, Italy
Tel.: +39-049-8213505

Fax: +39-049-8213502

E-mail: barbato@child.pedi.unipd.it

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Bronchial asthma in childhood is a disease that often starts in children when under 3 years of age and persists as they grow up (1). Its prevalence is increasing (2) and therefore a better understanding is needed for a more adequate diagnosis and treatment, especially in younger children.

New research opportunities have become available with the introduction of fiberoptic bronchoscopy (FB) and bronchoalveolar lavage (BAL), which are being used to an increasing extent in pediatric pneumology centres (3).

There are reports of high levels of eosinophils in the BAL collected from asthmatic children (4). Little is known, however, about the role of neutrophils in the chronic inflammation induced by bronchial asthma, described in recent studies on adults (5,6) and children (7), as cells that remain activated in asthma disease.

The aim of the present study was to contribute towards a better understanding of this aspect of asthmatic inflammation.

Subjects and methods

Subjects

As, for ethical reasons, we could not study the cell profiles and mediators in the BAL fluid of children with asthma, we performed a retrospective study in allergic children with asthma (8) who had undergone FB and BAL for other indications (3).

The study included 13 asthmatic children (seven males, six females; mean age 7 years 8 months, range 3 years 3 months to 17 years 7 months) who had been hospitalized in the Department of Pediatrics, Padova from December 1997 to May 1999 to undergo FB for the following indications: eight for recurrent chest radiographic shadowing

in the same lobe (four in the middle lobe, three in the lower left lobe, and one in the upper left lobe) – which dated from an average of 3 months (range 1–6 months) before FB and BAL – two for persistent atelectasis, and three for chronic cough. The diagnosis of asthma was based on the National Heart Lung and Blood Institute (NHLBI) and World Health Organization (WHO) guidelines (9). Seven children suffered from intermittent asthma, reporting symptoms less than twice a week, night-time symptoms less than twice a month, and completely symptom-free periods between exacerbations. The symptoms were controlled by inhaled bronchodilators. Six children suffered from mild-to-moderate persistent asthma with symptoms occurring more than twice a week and/or once a week at night, and needed continuous inhaled steroid administration (the average dose of beclomethasone dipropionate was 586 µg/day, range 500–750 µg) up until the day when FB and BAL were performed.

All children were skin-prick test or radioallergosorbent test (RAST) positive for inhaled allergens.

Nine children underwent spirometry (flow-volume curves were measured by using the Pulmonet-III, Sensor Medics, CA, USA) (Table 1), which documented varying degrees of airflow obstruction (that improved both spontaneously and after beta-2 inhalation).

All children were immunocompetent and none had laboratory signs of acute infection or were receiving antibiotic treatment at the time of hospitalization.

The control group comprised 16 children (nine females and seven males; mean age 8 years 10 months, range 3 years 6 months to 16 years 3 months), who underwent bronchoscopic BAL during elective surgery for non-pulmonary disease (10 for adenoidectomy and/or tonsillectomy, six for minor surgery). As medical examination prior to surgery revealed no clinical history of asthma or atopic disease, these children underwent no allergy tests. Informed parental consent was obtained for all children taking part in the study.

FB and BAL

The FB and BAL were performed by the same operator using an Olympus 3C30 or P10 fiberoptic bronchoscope, depending on whether the children were younger or older than 9 years of age. During the procedure, patients were sedated with intravenous (i.v.) propofol (3 mg/kg) and atropine was used for premedication. Local anesthesia was induced in the lower airways

with lidocaine 0.5%. BAL was performed in the lung segment that, judging from endoscopic examination and the most recent chest X-ray, appeared to be most greatly affected by the inflammatory process; failing this indication, BAL was performed in the middle lobe. The amount of saline solution injected for each child was 1 ml/kg of body weight and was divided into three aliquots of 5–20 ml, depending on the weight of the patient (10); the first fraction was used for culture and the other two were pooled (11) and sent to the laboratory for analysis of total and differential cell counts, and for assay of myeloperoxidase (MPO) and eosinophil cationic protein (ECP) levels.

Cytological analysis and mediator assay

Before assessing the cellular and non-cellular components, the fluid was centrifuged at 30×g for 10 min. The supernatant was used for assay of MPO and ECP levels by radioimmunoassay (Pharmacia-Upjohn, Uppsala, Sweden); results were expressed in µg/l. The lower limit of detection for ECP was 1 µg/l and for MPO it was 40 µg/l.

After eliminating the supernatant, the cell pellet was suspended in 1 ml of Hank's balanced salt solution (Ca²⁺- and Mg²⁺-free) and centrifuged (Phicom Co., Cheshire, UK), spinning $\approx 1 \times 10^6$ cells/ml. The centrifuged cell preparations were air-dried and stained with May Grunwald-Giemsa stain.

Cell evaluation was performed by the same investigator, blinded to the patients' clinical details, using a light microscope (Leitz-Dialux2, Ernst Leitz, Weitzlar, Germany). At least 1000 cells were examined and 100 were counted for each patient. A small sample of cell suspension was used for evaluation of total cell counts, using the Coulter-Z1 hemocytometer.

The total cell values and the different cell populations were expressed as $\times 10^3$ /ml.

Statistical analysis

Age was expressed as mean and range, forced expiratory volume in 1 s (FEV_1) as a percentage of the reference values, and total immunoglobulin E (IgE) as median and range.

The amount of BAL fluid obtained (expressed as a percentage of the instilled saline solution) is presented as mean $\pm$ SD. The total cell and differential cell counts are expressed per ml of fluid recovered and shown as medians and ranges. ECP and MPO are expressed as medians and ranges. The non-parametric Mann-Whitney Rank Sum test was used to compare results of

Table 1. Clinical features of asthmatic patients

Subject no.	Gender	Age	FEV ₁ (%)	Total IgE (IU/ml)	Allergy	Inhaled steroid
1	M	3 y 3 m	—	30	G	No
2	M	11 y 6 m	92	504	HDM	Yes
3	F	6 y 1 m	105	472	HDM	Yes
4	F	7 y 11 m	93	1575	HDM	Yes
5	M	17 y 7 m	89	1172	G	No
6	M	8 y 3 m	97	121	C	No
7	M	7 y 2 m	87	32	HDM	Yes
8	F	3 y 11 m	—	8	B	No
9	F	10 y 2 m	90	252	A	No
10	F	9 y 11 m	86	489	C	Yes
11	M	8 y 2 m	104	418	HDM	Yes
12	F	5 y 5 m	—	94.3	A	No
13	F	4 y 9 m	—	306	C	No
		7 y 8 m*		306‡		
		(3 y 3m–17 y 7 m)†		(8–1575)†		

A, alternaria; B, betulla; C, cat; HDM, house dust mites; G, grasses.

FEV₁, forced expiratory volume in 1 s; IgE, immunoglobulin E; m, months; y, years.

*Mean.

†Range.

‡Median.

asthmatics and controls. The same test was used to compare the intra-group results between patients with intermittent or persistent asthma.

The correlation between the number of neutrophils and MPO was obtained using Pearson's correlation coefficient.

A probability value of <0.05 was considered statistically significant.

Results

The total and differential cell counts in the BAL fluid from the asthmatic children and controls are shown in Table 2.

Total cell counts were much higher in the asthmatic children (median $1314 \times 10^3/\text{ml}$) than in the controls (median $625 \times 10^3/\text{ml}$), $p = 0.08$. The numbers of eosinophils, lymphocytes, and epithelial cells were not significantly different statistically between the two groups of children, whereas the neutrophil levels were significantly

higher in the asthmatics (median $80 \times 10^3/\text{ml}$) than in the controls (median $26 \times 10^3/\text{ml}$), $p = 0.02$. ECP levels were higher in the asthmatics (median $24 \mu\text{g/l}$) than in the controls ($2 \mu\text{g/l}$), $p < 0.001$, as were the MPO values (median $189 \mu\text{g/l}$ in asthmatics vs. $40 \mu\text{g/l}$ in controls, $p < 0.001$).

When the neutrophil counts and the corresponding values of MPO were analyzed in asthmatic children, a linear correlation was found with an R_s of 0.73 ($p < 0.005$). No significant correlation was found between ECP values and eosinophil counts ($r = -0.10$; $p = 0.74$); a positive trend was found between ECP values and neutrophil counts ($r = 0.50$; $p = 0.07$).

When the group with asthma was divided according to disease severity (six patients had mild-to-moderate persistent asthma and seven patients had intermittent asthma), neutrophil counts ($p = 0.06$), ECP values ($p = 0.06$) and

Table 2. Cell population and eosinophil cationic protein (ECP) and myeloperoxidase (MPO) values in bronchoalveolar lavage (BAL) of asthmatic children and controls

	Asthmatics	Controls	p-value
No. of patients	13	16	
BAL recovered (%)	43 ± 10.8	37.7 ± 10.7	0.32
Total cell count* ($\times 10^3/\text{ml}$)	1314 (84–4000)	625 (144–1167)	0.08
Alveolar macrophages* ($\times 10^3/\text{ml}$)	714 (51–1890)	542 (225–2088)	0.79
Neutrophils* ($\times 10^3/\text{ml}$)	80 (12–1191)	26 (0–144)	0.02
Eosinophils* ($\times 10^3/\text{ml}$)	0 (0–800)	0 (0–42)	0.13
Lymphocytes* ($\times 10^3/\text{ml}$)	75 (0–960)	21 (0–84)	0.08
Epithelial cells* ($\times 10^3/\text{ml}$)	5 (0–280)	6 (0–144)	0.41
ECP* ($\mu\text{g/l}$)	24 (2–180)	2 (2–10)	<0.001
MPO* ($\mu\text{g/l}$)	189 (40–5500)	40 (40–85)	<0.001

*Values are expressed as median and ranges (in brackets).

Table 3. Cell population and eosinophil cationic protein (ECP) and myeloperoxidase (MPO) values in bronchoalveolar lavage (BAL) in children with mild-to-moderate persistent asthma (six patients) and in children with intermittent asthma (seven patients)

	Persistent asthma	Intermittent asthma	p-value
No. of patients	6	7	
Total cell count ($\times 10^3/\text{ml}$)	1135 (571–3000)	850 (84–4000)	0.52
Alveolar macrophages* ($\times 10^3/\text{ml}$)	588 (51–1890)	714 (67–1400)	0.77
Neutrophils* ($\times 10^3/\text{ml}$)	282 (48–1191)	58 (12–840)	0.06
Eosinophils* ($\times 10^3/\text{ml}$)	27 (0–120)	0 (0–800)	0.62
Lymphocytes* ($\times 10^3/\text{ml}$)	50 (0–510)	78 (0–960)	0.47
Epithelial cells* ($\times 10^3/\text{ml}$)	51 (0–280)	5 (0–96)	0.37
ECP ($\mu\text{g}/\text{l}$)	84.5 (12–180)	14 (2–28)	0.06
MPO ($\mu\text{g}/\text{l}$)	614 (40–5500)	46 (40–239)	<0.04

*Values are expressed as median and ranges (in brackets).

MPO values ($p < 0.04$) were significantly higher in the group with mild-to-moderate persistent asthma, while no significant differences were observed in the total number of alveolar macrophages, eosinophils, lymphocytes, and epithelial cells (Table 3 and Fig. 1). The mean age of patients in the group with intermittent asthma did not differ significantly from those of the persistent asthma group (mean ages 101.5 months and 91.4 months, respectively) confirming that the results obtained were unlikely to depend on the children's age or the size of bronchoscope used.

In our series, the indication for FB was recurrent chest radiographic shadowing in same lobe in eight patients; four of these patients had intermittent asthma and four had persistent asthma.

Six BAL cultures (four in patients with intermittent asthma and two with persistent disease) were positive for bacterial growth. The cultures from the four children with intermittent asthma were positive for *Haemophilus influenzae* (three patients) and *Streptococcus pneumoniae* (one patient); the two positive cultures from children with persistent asthma were positive for *S. pneumoniae* in one and for

H. influenzae in the other. No differences were found among the cell counts and mediator values of these six patients and the patients with a negative BAL culture.

No differences were found between the cell counts and mediator values of patients with recurrent chest radiographic shadowing in same lobe and those with persistent atelectasis and chronic cough.

Discussion

A major constraint on BAL studies in children with asthma is the ethical difficulty of using investigative procedures that carry some degree of discomfort. There is consequently evidence of substantial benefit only when a complication of asthma justifies the use of FB and BAL. This limitation means that only selected asthmatic children can be studied using BAL.

Our asthmatic patients had higher total cell count than controls, higher levels of neutrophils and MPO (a mediator that expresses their activation), and higher levels of ECP with no significant difference in the number of eosinophils. The higher total cell count in the asthmatic patients than in the controls is even more significant if we consider that the total cell count observed in the BAL of our controls is higher than reported elsewhere (11,12), although not significantly so – the slight increase might be explained by the fact that 10 of our controls underwent BAL during adenotonsillectomy for adenotonsillar hypertrophy with narrowing of the upper airways.

The inflammation caused by asthma explains the higher ECP levels. An increase in ECP and eosinophils in the BAL of asthmatic children after non-specific bronchial challenge with histamine has been reported (4). More recently, high ECP levels were also recorded in the BAL of asthmatic children without bronchial stimulation and during relatively asymptomatic

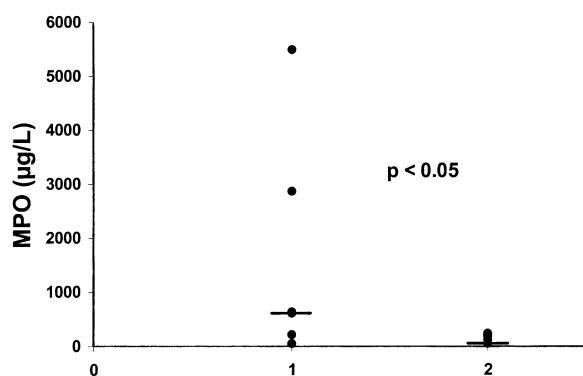


Fig. 1. Myeloperoxidase (MPO) values in six children with mild-to-moderate persistent asthma (1) and in seven children with intermittent asthma (2).

periods (13). In our study of asthmatic children without any non-specific bronchial stimulation, we found high ECP levels with no increase in the eosinophil count. This is consistent with a recent report (14) demonstrating in biopsy specimens that the severity of bronchial inflammation is greater in persistent asthma than in intermittent asthma.

The inflammation caused by asthma could also explain the rise in neutrophil levels. Neutrophil activation has already been reported in asthmatic adults (5,6) and also, more recently, in asthmatic children, and it is related to symptom severity (7). In our opinion, our findings were not influenced by the positive BAL culture, given that positivity was also found in four children with intermittent asthma (with lower neutrophil and MPO counts) and in only two children with persistent asthma, who had higher neutrophil and MPO levels. The positive trend between ECP values and neutrophil counts observed in our patients is in agreement with the relationship previously reported in adults, where raised levels of ECP and neutrophils, but not eosinophils, were specifically associated with severe, persistent disease (15). The relationship between ECP values and neutrophil number could be explained by the fact that ECP might reflect eosinophil turnover, while neutrophil phagocytosis of eosinophil granules could lead to an underestimation of eosinophil counts (16).

In our asthmatic patients these results demonstrate an ongoing neutrophil inflammation that is more relevant in children with mild-to-moderate persistent asthma than in patients with intermittent asthma, while the eosinophil activity (in terms of number and mediator production) does not seem to differ substantially between the two groups of asthmatic patients.

In conclusion, our findings demonstrated that more severe asthma, requiring the use of inhaled steroids to control the patient's symptoms, is correlated with a persistent, neutrophil-mediated inflammation.

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