

# Evaluation of the activity of eosinophils in the blood of very low birth weight infants

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## Summary

**Background:** Eosinophilia is a common finding in neonatal units. The role of eosinophilia in preterm infants is uncertain. The aim of the study was the assessment of absolute eosinophil count (AEC) and eosinophil cationic protein (ECP) concentration in the blood of VLBW infants during the first 4 weeks of life.

**Material and Methods:** Prospective study in the University Hospital NICU included 58 preterm infants with gestational age 24–34 weeks ( $x \pm SEM$ :  $28.8 \pm 0.3$ ) and mean birth weight  $1127 \pm 31$  g. AEC, ECP and total IgE levels at admission, and at the age of 10, 20 and 30 days of life were measured.

**Results:** There were no correlations between AEC and gestational age ( $R=0.14$ ,  $p=0.33$ ) or birth weight ( $R=0.13$ ,  $p=0.34$ ), and between ECP and gestational age ( $R=-0.05$ ,  $p=0.8$ ) or birth weight ( $R=0.11$ ;  $p=0.53$ ) either. There were significant correlations between AEC and ECP at admission ( $R=0.46$ ;  $p=0.001$ ) and after the 5<sup>th</sup> day of life ( $R=0.51$ ;  $p<0.001$ ). AEC  $> 1500/mm^3$  was observed in 26 (44%) children. Significant eosinophilia was recognized on average on the 24<sup>th</sup> day of life. Only 3 children had positive total IgE levels ( $>2kU/l$ ). There were no correlations between AEC or ECP and total IgE levels.

**Conclusions:** There is a correlation between AEC and ECP confirming that an increased number of eosinophils in preterm infants is connected with increased activity of eosinophils.

**Key words:** VLBW infants • eosinophilia • eosinophil cationic protein • ECP • immunoglobulin E • IgE

## BACKGROUND

Maturation of eosinophils takes place in bone marrow. After 5–6 days of maturation, eosinophils pass to the bloodstream. In the blood eosinophils circulate for only 3–8 hours and enter the tissues. A mature eosinophil cell is 10–15  $\mu m$  large, with characteristically divided nucleus and multiple granules. There are two types of eosinophils with different activity. In healthy subjects, 90% of all circulating eosinophils are non-active eosinophils [1]. In patients with atopy, the number of activated eosinophils increases and amounts up to 60 percent [2]. The pathophysiologic role of eosinophils is connected with delivered mediators. There are two types of mediators: released from cytoplasmic granules and produced *ad hoc* during prolonged activation. According to the recent studies, cytoplasmic granules mediators which play the main role in the pathophysiologic processes are: MBP – main basic protein, EPO – eosinophil peroxidase, ECP – eosinophil cationic protein, EDN- eosinophil-derived

neurotoxin (which is also called EPX – eosinophil protein X). The mediators produced during prolonged activation include: leukotrien C4, platelet activating factor (PAF) and cytokines (IL-1a, IL-3, IL-5, TNF- $\alpha$ , GM-CSF) [1].

ECP and EPX are clinically important and easily measurable mediators. ECP possesses strong cytotoxic activity, but it can also decrease the proliferation of lymphocytes T [3,4].

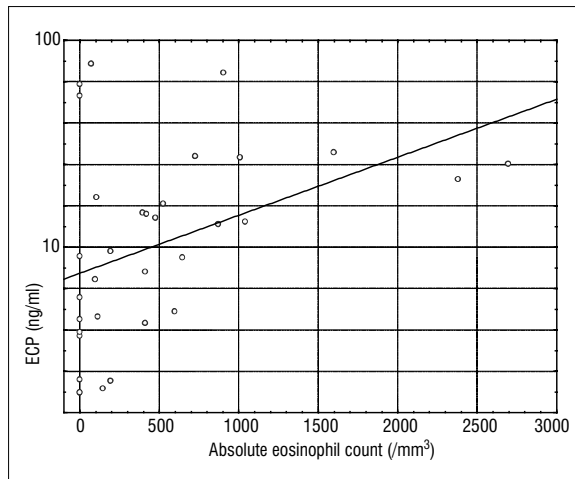
An increased ECP concentration was demonstrated in patients with bronchial asthma [5,6]. There is a clear correlation between severity of the disease and ECP concentration. Moreover, ECP concentration can be used for monitoring severity of asthma and response to treatment [7,8].

An increased ECP concentration and an increased number of eosinophils were also observed in infants with respiratory syncytial virus infection and increased ECP con-

**Table 1.** Baseline characteristics of study participants\*.

Birth weight, mean (SEM)	1127 g (31)	
Gestational age, mean (SEM)	28.8 weeks (0.3)	
Female	28	(48)
Vaginal delivery	25	(43)
Small for gestational age	7	(12)
Mechanical ventilation at admission	41	(71)

\* expressed as a number (percentage) of patients unless otherwise indicated



**Figure 1.** Correlation between absolute eosinophil count and concentration of eosinophil cationic protein at admission.

centration was used as a predictor of future asthma development [9–12].

Eosinophilia is a common finding in neonatal units [13], but the role of eosinophilia in infants is uncertain. Moreover, there is no available data evaluating activity of eosinophils in preterm infants. Additionally, the data analyzing correlation between eosinophilia in early infancy and future development of allergy is insufficient. Prematurity is an important risk factor of chronic lung disease and hyperreactivity of airways. There exists a hypothesis stating that eosinophil activation observed in neonates can be a sign of a specific way in which the child responds to stimulation, the way which can predict the development of asthma in future life.

The aim of the study was the assessment of absolute eosinophil count (AEC) and eosinophil cationic protein concentration in the blood of very low birth weight infants during the first 4 weeks of life.

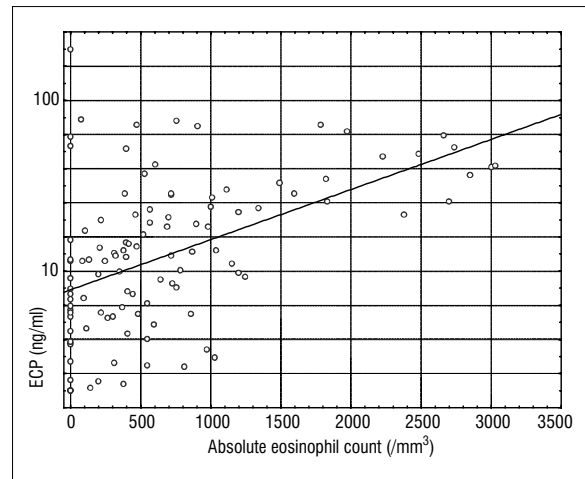
**MATERIAL AND METHODS**

**Subjects**

Over the period of 18 months (June 2001 – December 2002) 58 newborns were recruited to the study. Inclusion criteria were as follows: (a) birthweight (1500 g, (b) age at admission <72 hours. The only exclusion criteria were early sepsis and multiple congenital malformations.

**Table 2.** Laboratory data at admission.

	Median	Mean	SEM	Range
WBC (/mm <sup>3</sup> )	10,600	14,250	1,200	2,700–64,600
Eosinophils (%)	1.5	1	0.2	0–8
Absolute eosinophil count (/mm <sup>3</sup> )	100	188	37	0–2380
ECP concentration (ng/ml)	5	16.5	6.3	2–77.8



**Figure 2.** Correlation between absolute eosinophil count and concentration of eosinophil cationic protein after the 5<sup>th</sup> day of life.

**Intervention**

At admission, and at the age of 10<sup>th</sup>, 20<sup>th</sup> and 30<sup>th</sup> day of life AEC, ECP and total IgE levels were evaluated. ECP concentration was analyzed by radioimmunoassay (Pharmacia, Upsala, Sweden).

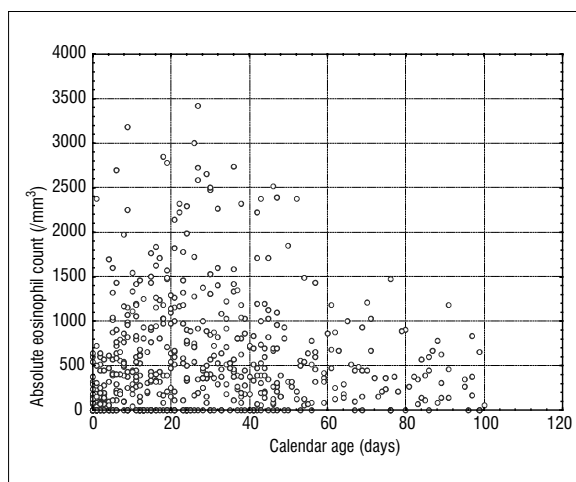
**Statistical analysis**

Categorical variables were statistically analyzed using a chi-square test or a Fischer exact test. Continuous variables were analyzed using a t-student test or Mann-Whitney U-test. Statistica for Windows statistical software version 6.0PL (StatSoft, Inc.2001) was used for the analyses, and p<0.05 was considered statistically significant.

**RESULTS**

Basic characteristic of the studied infants is presented in Table 1. Birth weight of 22 (38%) newborns was less than 1000 g. At admission, AEC and ECP were evaluated in all patients (Table 2.). In infants admitted after 1.02.2002 total IgE level was also analyzed.

At admission, there were no correlations between AEC and gestational age (R=0.14, p=0.33) or birth weight (R=0.13, p=0.34). Additionally, there were no correlations between ECP and gestational age (R=-0.05, p=0.8) or birth weight (R=0.11; p=0.53).



**Figure 3.** Correlation between absolute eosinophil count and calendar age of studied infants.

AEC (Me: 0 vs 80 /mm<sup>3</sup>,  $p=0.8$ ) and ECP concentrations (Me: 13.7 vs 9.7 ng/ml;  $p=0.5$ ) were similar in the groups of newborns: 1) with mechanical ventilation and 2) without mechanical ventilation.

There were significant correlations between AEC and ECP at admission ( $R=0.46$ ;  $p=0.001$  – Figure 1.) and after 5<sup>th</sup> day of life ( $R=0.51$ ;  $p<0.001$  – Figure 2.).

During the study 666 complete blood counts were performed. Significant eosinophilia (AEC >1500/mm<sup>3</sup>) was observed in 26 (44%) children. In 8 cases eosinophilia was recognized on more than 2 occasions, but in 16 infants it was detected only once. Significant eosinophilia was observed (Me) on the 24<sup>th</sup> day of life. The correlation between absolute eosinophil count and the calendar age of studied infants is presented in Figure 3. The comparison of selected variables between patients with and without significant (>1500/mm<sup>3</sup>) eosinophilia is presented in Table 3.

Only 3 children had positive total IgE levels (>2kU/l). There were no correlations between AEC or ECP and total IgE levels.

## DISCUSSION

Bhat et al. in 1981 recognized that 75% preterm infants had an absolute eosinophil count higher than 700/mm<sup>3</sup> [14]. Moreover, Sharma et al. noted that the prevalence of eosinophilia is higher in extremely preterm newborns [15]. Our results confirmed these observations. Significant eosinophilia (>1500/mm<sup>3</sup>) was recognized in 44% infants, with high incidence between the 20<sup>th</sup> and 40<sup>th</sup> day of life. Interestingly, there were no correlations between AEC and gestational age or birth weight.

The analysis of risk factors of eosinophilia in preterm neonates was performed by Fayon et al. [16]. The authors noted that the risk of eosinophilia is higher in infants receiving multiple red blood transfusions, prolonged parental nutrition or mechanical ventilation.

**Table 3.** Comparison of selected variables between patients with and without significant (>1,500/mm<sup>3</sup>) eosinophilia.

	Patient with significant eosinophilia (n=26)	Patients without significant eosinophilia (n=32)
Birthweight, mean (SEM), g	1092 (36)	1155 (37)*
Gestational age, mean (SEM), wks.	28.4 (0.36)	29 (0.37)*
Female	12 (46)	16 (50)**
Vaginal delivery	10 (40)	15 (47)**
Small for gestational age	2 (8)	5 (15)**
Mechanical ventilation at admission	20 (77)	21 (67)**
Bronchopulmonary dysplasia	9 (35)	9 (28)**

Expressed as a number (percentage) of patients unless otherwise indicated.

There were no differences between the groups by:

\* t-student test; \*\* chi-square test

Until now, there is no explanation for such a coincidence.

Other results were presented by Ehara et al. [17]. The authors analyzed correlation between medical procedures (mechanical ventilation, antibiotics use, vein catheterization, type of feeding) and prevalence of eosinophilia. Only a correlation between erythropoietin treatment and AEC was observed. But, all available papers discussed only eosinophil count, not mentioning the activity of eosinophils.

In the present paper, we analyzed eosinophil activity by evaluating eosinophil cationic protein concentration in the blood. We did not find any correlation between ECP concentration and gestational age and birthweight. Neither mechanical ventilation or asphyxia influenced ECP concentrations. However, we have found a significant correlation between AEC and ECP at admission and after the 5<sup>th</sup> day of life. These observations confirm that the increased number of eosinophils in preterm infants is connected with the increased activity of eosinophils. There were no correlations between AEC or ECP and total IgE levels, which can suggest that the activation of eosinophils in neonates is not connected with IgE production, and eosinophilia in neonates does not predict the development of atopy.

The ECP and EPX concentrations in the nasal aspirates in the 1<sup>st</sup> month of life were evaluated in prospective, multicenter SPACE study [18]. The authors recognized higher ECP and EPX concentrations in infants with positive family history for atopy.

Yamamoto et al. [19] performed a study comparing 10 neonates with severe bronchopulmonary dysplasia and 7 newborns with other respiratory failure. The ECP concentrations both in the blood and tracheal aspirates were significantly higher in babies with BPD. The results mentioned above were confirmed by Raghavender et al. [20]. The authors suspected that the increased number of eosinophils in preterm infants was connected with the increased activity of eosinophils, which is also related to

the increased risk of developing bronchopulmonary dysplasia. Our results did not confirm such observation, but we measured ECP concentration in the blood only.

### CONCLUSIONS

1. There is no correlation between AEC and gestational age or birth weight. Additionally, there is no correlation between ECP and gestational age or birthweight.
2. Absolute eosinophil count is changing during the first 2 months of life.
3. Risk factors of eosinophilia in preterm infants are uncertain.

There is a correlation between AEC and ECP confirming that the increased number of eosinophils in preterm infants is connected with the increased activity of eosinophils.

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