Neonatology

Predictive Value of Eosinophilia and Basophilia in the Diagnosis of Bronchopulmonary Dysplasia in Premature Infants

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ABSTRACT

OBJECTIVE: To compare the presence of eosinophilia and basophilia between two groups with and without bronchopulmonary dysplasia in preterm infants and, to investigate whether there was a correlation between the bronchopulmonary dysplasia severity and degree of eosinophilia.

STUDY DESIGN: In this retrospective cohort study, we have evaluated premature babies who were admitted between 2007 and 2017 to the Neonatal Intensive Care Unit of Hacettepe Hospital. Hospital records were scanned and 85 preterm infants diagnosed with bronchopulmonary dysplasia formed the study group while 89 babies without bronchopulmonary dysplasia formed the control group. Necessary demographic, neonatal, and laboratory data were withdrawn from the electronic registry and patient files.

RESULTS: In the study group, there was a statistically significant difference in the severity of eosinophilia and basophilia in the first day, first week, second week, third week, and fourth week compared to the control group ($p<0.05$). According to the mixed effect model, after adjusting results for gestational age and birth weight there was a statistically significant difference between groups in terms of eosinophil and basophil values ($p<0.05$).

CONCLUSION: The number of eosinophils and basophils increased in patients with bronchopulmonary dysplasia without an increase in the number of leukocytes.

Keywords: Basophilia, Bronchopulmonary dysplasia, Eosinophilia Infant, Newborn, Premature

Introduction

Bronchopulmonary dysplasia (BPD), also known as chronic lung disease in premature infants, is an important respiratory disease resulting in morbidity and mortality in preterm infants (1). Bronchopulmonary dysplasia is caused by an imbalance between lung damage and repair in the developing immature lung. Alveolar simplification and dysmorphic pulmonary vascularization are the histopathological characteristics in most babies with BPD (2).

In infants in the neonatal intensive care unit, especially premature, it is likely to detect eosinophilia in full blood count analysis. As the gestational age decreases, the rate of detection of eosinophilia increases further (3,4). Although it has been associated with some conditions such as infection, parenteral nutrition or a family history of atopic eczema. The cause and pathogenesis of eosinophilia in newborns are not very clear (5,6).

Eosinophilia is a common hematologic finding in preterm infants. The incidence and severity of eosinophilia increase as the gestational week decreases (4). In studies evaluating eosinophilia and BPD relationship; eosinophilia has been found to continue in weekly analysis starting from the first week (3,5).

Basophilia is a rare hematologic finding in complete blood counts. Basophilia often coexists with eosinophilia. Basophilia is the condition of having the number of basophils as 200 cells/uL or above (7). There are no studies in the literature comparing basophil counts in premature infants with and without bronchopulmonary dysplasia.

In this study, we planned to retrospectively compare the

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presence of eosinophilia and basophilia in preterm infants (gestational age 34 weeks or below) with and without BPD and, to investigate whether there was a correlation between the BPD phase and the severity of eosinophilia and basophilia.

Material and Method

Patients

The retrospective study included premature infants who were admitted between 2007 and 2017 to the Neonatal Intensive Care Unit of Hacettepe University. Ethical approval was obtained from Hacettepe University Clinical Research Ethics Committee (ethics committee's reference number: GO 17/40-06) before the study. All procedures were performed according to the Declaration of Helsinki. Hospital records were scanned and 137 babies diagnosed with BPD and 149 babies non-BPD were identified after having excluded major congenital anomaly (congenital heart disease, lung anomaly, congenital diaphragmatic hernia, etc.), chromosomal anomaly, metabolic disease, hypoxic-ischemic encephalopathy and, infants with perinatal hypoxia. Among patients with bronchopulmonary dysplasia, 13 patients were not included in the study due to incomplete data in file records and 39 patients due to incomplete data on complete blood count and/or peripheral smear results. The study group consisted of 85 infants with complete study data. 22 of the babies included in the control group were not included in the study due to incomplete data on file records and 38 of the patients in the control group were not included in the study due to incomplete data on full blood count and/or peripheral smear results. The remaining 89 babies formed the control group (The flowchart is given in Figure 1).
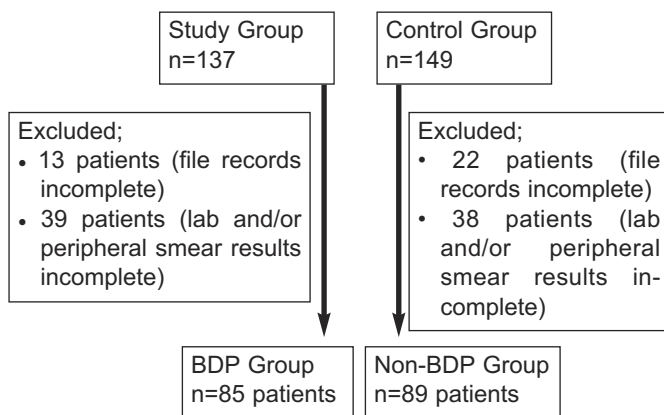

Figure 1: The Study Flowchart

Demographic, neonatal findings, and hematologic laboratory parameters of the patients included in the study were recorded.

In our hospital, blood samples that are taken to tubes containing ethylenediaminetetraacetic acid (K2-EDTA) are analyzed at the Unicel DxH 800 (Beckman Coulter, Brea, CA, USA) automatic cell counter. The study data were retrospectively recorded from the automatic full blood count reports. In our hospital, peripheral blood smear examination is performed simultaneously with all complete blood count analyses of newborns and cell number and percentage ratios are evaluated by counting 100 cells. Retrospectively, the automatic complete blood count samples were compared with the simultaneous peripheral smear reports. The samples in which the peripheral smear results do not comply with automatic counting, and those which contained normoblast, and those that could not be evaluated because of the poor propagation quality, were excluded from the study.

Diagnosis of Bronchopulmonary Dysplasia

The diagnosis of bronchopulmonary dysplasia of the patients in the study group was made according to the criteria in Table I.

Table I: Definition of bronchopulmonary dysplasia: diagnostic criteria (8)

Gestational age	< 32 wk.	≥ 32 wk.
Timepoint of assessment	36 wk. PMA or discharge to home, whichever comes first	>28 d but <56 d postnatal age or discharge to home, whichever comes first
	Treatment with oxygen >21% for at least 28 d plus	
Mild	Breathing room air at 36 wk. PMA or discharge, whichever comes first	Breathing room air by 56 d postnatal age or discharge, whichever comes first
Moderate	Need for <30% oxygen at 36 wk. PMA or discharge, whichever comes first	Need for <30% oxygen at 56 d postnatal age or discharge, whichever comes first
Severe	Need for >30% oxygen and/or positive pressure, (PPV or NCPAP) at 36 wk. PMA or discharge, whichever comes first	Need for <30% oxygen at 56 d postnatal age or discharge, whichever comes first

BPD: Bronchopulmonary dysplasia, NCPAP: Nasal continuous positive airway pressure, PMA: Postmenstrual age, PPV: Positive-pressure ventilation

Definition of eosinophilia

The count of eosinophils is 700 cells/mm3 or above is defined as eosinophilia. The number of eosinophils is defined as mild eosinophilia between 700-999 cells/mm3, moderate eosinophilia between 1000-2999 cells/mm3, and severe eosinophilia of 3000 cells/mm3 and above (8).

Definition of basophilia

Basophilia is accepted as the number of basophils is 200 cells/μL and above (7).

Definition of sepsis

Infants with clinical signs and symptoms that cannot be

explained by a diagnosis of a disease other than sepsis and who scored 10 or more in Töllner scoring (9) were evaluated as sepsis and complete blood count, peripheral blood smear, C-reactive protein, procalcitonin, blood culture, chest x-ray if necessary, urinary culture, cerebrospinal fluid (CSF) culture if necessary are taken and empirical antibiotic (ampicillin-gentamycin) treatment is started. In the follow-up, if there is bacterial-fungal growth in blood and/or sterile body fluids, a definitive diagnosis was made as culture-proven sepsis. Patients who had a blood count taken at least three days before sepsis and/or at least seven days after the diagnosis of sepsis and those who did not have bacterial reproduction in the last blood and/or sterile body fluid culture (urine, CSF) were included in the study (10,11).

Statistical Analysis

The normality of distribution of continuous variables was tested by Shapiro-Wilk Test. Mann-Whitney U test (for non-normal distributing data) was used for the comparison of two independent groups and the Chi-square test was used to assess the relationship between categorical variables. A mixed-effect model was applied to evaluate the effect of time, group, group time interaction, gestational week, and birth weight on the change in eosinophil, basophil, and leukocyte values.

In mixed-model individuals were considered as random factors and groups and time were fixed factors. Unstructured covariance structure was used to estimate relations to repeated measures. Statistical analysis was performed with SPSS for Windows version 24.0 and a *p*-value <0.05 was accepted as statistically significant.

Results

Demographic data of the study and control group were given in table II and neonatal properties in table III.

There was a difference in terms of gender distribution between the groups (*p*=0.036). The antenatal steroid ratio was statistically lower in the study group (*p*=0.002). The proportion of mothers who smoked during pregnancy was higher in the control group (*p*=0.001). BPD rate was found to be statistically high in patients with premature membrane rupture (*p*=0.002). The cesarean delivery rate was higher in the small for gestational age (SGA) infants in the study group (*p*=0.001 and *p*=0.047, respectively). In the study group, gestational week and birth weight were statistically lower than in the control group (*p*=0.001 and *p*=0.001, respectively) (Table II).

There was no statistical difference between groups in terms of respiratory distress syndrome (*p*=0.078). Drugs with increased eosinophil count among the side effects were examined; there was no statistical difference between the two groups in terms of fluconazole use (*p*=0.078), but the use of vancomycin and ibuprofen was significantly higher in the study group (*p*<0.005 and *p*<0.005, respectively) (Table III).

The frequency of sepsis and the need for intubation were statistically higher in the study group (*p*=0.001 and *p*=0.001, respectively) (Table III). In the patients in the study group, 5th minute Apgar scores and sepsis diagnosis day were statistically lower than in the control group (*p*=0.012 and p=0.001, respectively). Besides, mechanical ventilation duration (invasive and noninvasive), duration of oxygen requirement, antibiotic treatment, total parenteral nutrition, and duration of

Table II: Demographic data

		Study Group (n=85) n (%)	Control Group (n=89) n (%)	*p*
F/M		42/43 (49.4/50.6)	58/31 (65.2/34.8)	**0.036**
Use of antenatal steroids	(-)	47 (55.3)	28 (31.5)	**0.002**
	(+)	38 (44.7)	61 (68.5)	
Maternal smoking	Yes	1 (1.2)	16 (18.0)	**0.001**
	No	84 (98.8)	73 (82.0)	
Premature membrane rupture	Yes	20 (23.5)	6 (6.7)	**0.002**
	No	65 (76.5)	83 (93.3)	
Delivery	VD	73 (85.9)	89 (100.0)	**0.001**
	C/S	12 (14.1)	0 (0.0)	
Birth weight by gestational week	AGA	57 (67.1)	73 (82.0)	**0.047**
	SGA	27 (31.8)	16 (18.0)	
	LGA	1 (1.2)	-	
Gestational age (weeks)*		27 (26.4-28.1)	28 (27.4-29)	**0.001**
Birth Weight (gr)*		840 (670-930)	1050 (950-1170	**0.001**

* Median (25-75 percentile)

hospitalization were found to be statistically higher in the study group compared to the control group (p=0.001, respectively; p=0.001, p=0.001, p=0.001, p=0.001, and p=0.001) (Table III).

Table III: *Neonatal properties*

		Study Group (n=85) n (%)	Control Group (n=89) n (%)	*p*
		78 (91.8)	48 (53.9)	
Need for intubation	(+)	7 (8.2)	41 (46.1)	**0.001**
	(-)	69 (81.2)	62 (69.7)	
Respiratory distress syndrome	Yes	16 (18.8)	27 (30.3)	0.078
	No	16 (18.8)	27 (30.3)	
Fluconazole Use	No	69 (81.2)	62 (69.7)	0.078
	Yes	17 (20.0)	8 (9.0)	
Vancomycin Use	Yes	68 (80.0)	81 (91.0)	**0.001**
	No	32 (37.6)	15 (16.9)	
Ibuprofen Use	Yes	53 (62.4)	74 (83.1)	**0.001**
	No	78 (91.8)	44 (49.4)	
Sepsis	Yes	7 (8.2)	45 (50.6)	**0.001**
	No	4 (4.7)	0 (0.0)	
Pulmonary hypertension	Yes	81 (95.3)	89 (100.0)	**0.016**
	No	39 (45.9)	28 (31.5)	
Pneumonia	Yes	46 (54.1)	61 (68.5)	**0.051**
	No	61 (71.8)	88 (98.9)	
Hemodynamically significant patent Ductus Arteriosus	No	24 (28.2)	1 (1.1)	**0.001**
	Yes	56 (65.9)	85 (95.5)	
Intraventricular hemorrhage	No	12 (14.1)	4 (4.5)	**0.001**
	Grade I	17 (20.0)	0 (0.0)	
	Grade II-III-IV	65 (76.5)	81 (91.0)	
Retinopathy of prematurity	No	11 (12.9)	8 (9.0)	**0.001**
	Grade I	2 (2.4)	0 (0.0)	
	Grade II	7 (8.2)	0 (0.0)	
	Grade III	66 (77.6)	73 (82.0)	
Necrotizing enterocolitis	None	7 (8.2)	16 (18.0)	**0.002**
	Grade I	12 (14.2)	0 (0.0)	
	Grade II-III-IV	7 (8.2)	45 (50.6)	
Erythrocyte transfusion	(–)	78 (91.8)	44 (49.4)	**0.001**
	(+)	7 (7-8)	8 (7-9)	**0.001**
Apgar score at 5th*		19 (8-32)	2 (0-5)	**0.012**
Conventional MV duration (days)*		14 (6-20)	3 (2-4)	**0.001**
Non-invasive MV duration (days)*		57 (39-70)	10 (6-15)	**0.001**
Duration of oxygen support (days)*		2 (0-13)	7 (0-12)	**0.001**
Sepsis diagnosis day *		10 (6-14)	10 (7-10)	**0.001**
Antibiotic duration (days)*		31 (24-41)	12 (8-17)	**0.001**
Duration of caffeine Use (days)*		27 (21-38)	16 (12-22)	**0.001**
TPN duration (days)*		69 (53-81)	35 (26-42)	**0.001**
Duration of hospitalization (days)*				**0.001**

*Median (25-75 percentile), TPN: Total parenteral nutrition, MV: Mechanic ventilation

There was no statistical difference between groups in terms of incidence of pneumonia (p=0.051). In the study group, pulmonary hypertension, hemodynamically significant patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), erythrocyte suspension transfusion need was found to be statistically higher compared to the control group (p=0.016, p=0.001, p=0.001, p=0.002, p=0.001, and p=0.001, respectively) (Table III).

Of patients with BPD, 68 were classified as mild, 9 as moderate, and 8 as severe.

The first day, first week, second week, third week, and fourth-week values of leukocyte, eosinophil, and basophil in the study and the control group were given in Table IV.

There was no statistical difference between the study group and the control group in terms of leukocyte values of the first day, first week, second week, third week, and fourth week and basophil values of the first day (p=0.805, p=0.432, p=0.856, p=0.663, p=0.407, and p=0.895, respectively).

The first week, second week, third week, and fourth-week basophil values were found to be statistically higher in the study group compared to the control group (p=0.037, p=0.008, p=0.011, and p=0.001, respectively). There was a significant statistical difference in eosinophilia values between the groups in five different periods (p=0.001, p=0.001, p=0.001, p=0.001, and p=0.001, respectively) (Table IV).

Comparison of patients in the study group and control group based on the first day, first week, second week, third week, and fourth-week eosinophilia grades (mild, moderate, and severe) were given in Table V.

In the study group, there was a statistically significant difference in the severity of eosinophilia on the first day, first week, second week, third week, and fourth week compared to the control group (p=0.001, p=0.001, p=0.001, p=0.001, p=0.001, and p=0.001, respectively).

Since there was a statistical difference in gestational week and birth weight between the groups, eosinophil, and basophil values were compared after remedial analysis had been performed on gestational week and birth weight (Table VI and Table VII, respectively).

According to the mixed effect model, after adjusting results for gestational age and birth weight there was a statistically significant difference between groups in terms of eosinophil values (p=0.001) (Table VI).

After the effect of other factors was corrected, there was a statistically significant difference between the groups in terms of basophil values (p<0.005) (Table VII).

Eosinophil and basophil values of the patients after the remedial analysis were given in Figures 2 and 3, respectively.

Eosinophilia values according to BPD grades in the study group were given in table VIII.

There was a statistical difference between eosinophilia values in the first day of life, first week, second week, third week, and a fourth week among subgroups according to BPD grades (p=0.001, p=0.019, p=0.004, p=0.005, and p=0.049, respectively). In the study group, a right proportion was found between the BPD phase and the severity of eosinophilia.

Table IV: *Leukocyte, eosinophil, and basophil values of patients*

	Study group (n=85)*	Control group (n=89)*	*p*
Leukocyte count			
First day	9000 (6350-13600)	8100 (6900-11450)	0.81
First week	15700 (8050 -19900)	12200 (8450-18700)	0.43
Second week	16900 (9300-21900)	12100 (8600-18700)	0.86
Third week	11300 (9700-15700)	11300 (9400-13650)	0.66
Fourth week	9500 (8050-13000)	10400 (8350-13900)	0.41
Eosinophil count			
First day	700 (100-1050)	100 (52-265)	**0.001**
First week	870 (200-1150)	140 (90-200)	**0.001**
Second week	800 (300-1200)	200 (100-300)	**0.001**
Third week	900 (300-1250)	130 (80-395)	**0.001**
Fourth week	900 (500-1200)	300 (100-400)	**0.001**
Basophil count			
First day	200 (100-600)	150 (70-350)	0.90
First week	200 (100-765)	80 (30-130)	**0.037**
Second week	200 (100-615)	100 (70-110)	**0.008**
Third week	295 (135-670)	100 (75-380)	**0.11**
Fourth week	300 (110-650)	200 (100-300)	**0.001**

Median (25%-75%)

Table V: *Comparison of the study and the control groups based on the eosinophilia phases*

| Eosinophil count		Study group (n=85)	Control group (n=89)	p
		n (%)	n (%)	
First day	None	42 (49.4)	84 (94.4)	
	Mild	16 (18.8)	1 (1.1)	
	Moderate	23 (27.1)	4 (4.5)	**0.001**
	Severe	4 (4.7)	0 (0.0)	
First week	None	36 (42.4)	89 (100.0)	
	Mild	15 (17.6)	0 (0.0)	
	Moderate	26 (30.6)	0 (0.0)	**0.001**
	Severe	8 (9.4)	0 (0.0)	
Second week	None	35 (41.2)	88 (98.9)	
	Mild	15 (17.6)	0 (0.0)	
	Moderate	32 (37.6)	1 (1.1)	**0.001**
	Severe	3 (3.5)	0 (0.0)	
Third week	None	32 (37.6)	81 (91.0)	
	Mild	12 (14.1)	3 (3.4)	
	Moderate	35 (41.2)	5 (5.6)	**0.001**
	Severe	6 (7.1)	0 (0.0)	
Fourth week	None	32 (37.6)	80 (89.9)	
	Mild	19 (22.4)	5 (5.6)	
	Moderate	31 (36.5)	4 (4.5)	**0.001**
	Severe	3 (3.5)	0 (0.0)	

Table VI: *Eosinophil values after remedial analysis on gestational week and birth weight*

Parameter	Estimate	Std. Error	95% Confidence Interval		p
			Lower Bound	Upper Bound	
Intercept	2536.77	976.32	609.69	4463.83	0.01
BPD vs Control	654.74	161.05	337.17	972.31	**0.00**
Time					0.10
[time=1] vs 5	-113.93	121.94	-354.63	126.77	0.35
[time=2] vs 5	-157.86	112.42	-379.77	64.04	0.16
[time=3] vs 5	-117.07	94.29	-303.20	69.05	0.22
[time=4] vs 5	-61.12	99.12	-256.79	134.54	0.54
Gestational age	-67.24	40.21	-146.63	12.14	0.10
Birth weight	-0.27	0.27	-0.81	0.26	0.32

Median (25%-75%)

Table VII: *Basophil values after remedial analysis on gestational week and birth weight*

Parameter	Estimate	Std. Error	95% Confidence Interval		p
			Lower Bound	Upper Bound	
Intercept	999.80	443.78	123.78	1875.82	0.03
BPD vs Control	203.91	55.94	93.57	314.24	**0.00**
Time			.	.	0.59
[time=1] vs 5	28.03	87.28	-144.24	200.31	0.75
[time=2] vs 5	-102.98	61.73	-224.82	18.87	0.10
[time=3] vs 5	-70.73	63.89	-196.82	55.36	0.27
[time=4] vs 5	40.28	51.46	-61.29	141.85	0.44
Gestational age	-28.94	18.32	-65.11	7.22	0.12
Birth weight	0.025	0.12	-0.22	0.27	0.84

Table VIII: The relationship between BPD phases and the severity of eosinophilia

Eosinophil count	BPD phase			
	Mild * (n=68)	Moderate * (n=9	Severe * (n=8)	*p*
First day	200 (55-675)	1000 (900-2050)	1750 (1150-2400)	**0.001**
First week	500 (113-900)	1050 (835-1750)	1400 (1025-3425)	**0.019**
Second week	600 (200-875)	1100 (950-1200)	1250 (925-1925)	**0.004**
Third week	500 (200-1000)	1000 (900-1700)	1250 (1000-2925)	**0.005**
Fourth week	600 (315-1038)	900 (740-1650)	1500 (950-2125)	**0.049**
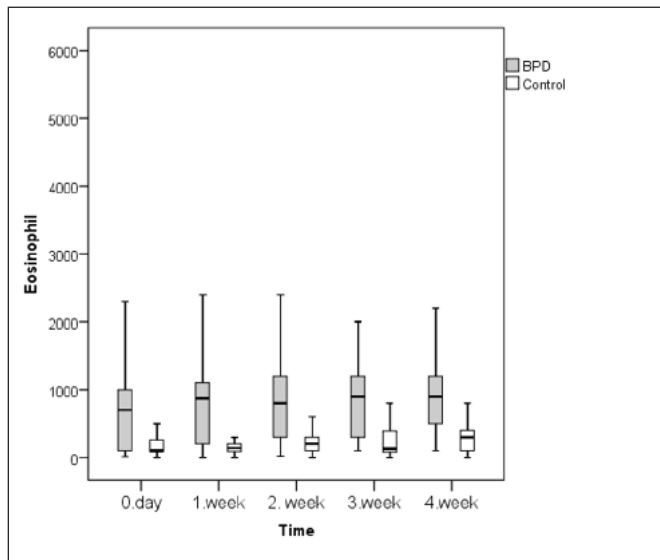
Figure 2: A graph of eosinophil value by group after correction on gestational week and birth week ($p0<0.05$; $p1<0.05$; $p2<0.05$; $p3<0.05$; $p4<0.05$).
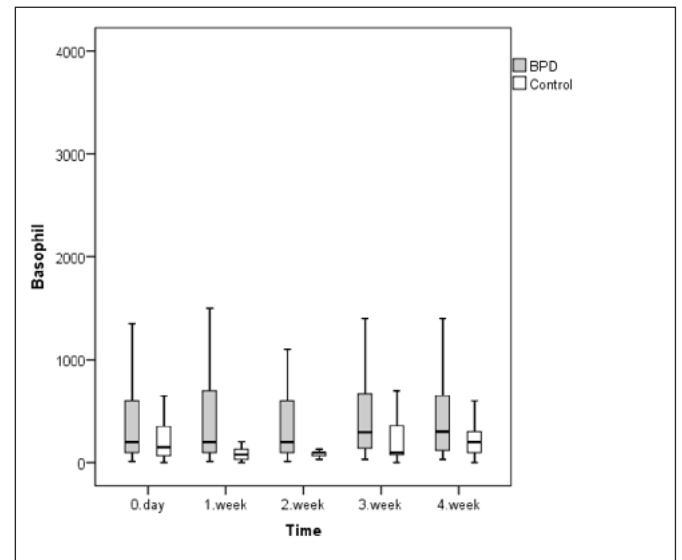
Figure 3: A graph of basophil value by group after correction on gestational week and birth week ($p0<0.05$; $p1<0.05$; $p2<0.05$; $p3<0.05$; $p4<0.05$).

Discussion

In recent years, due to advances in neonatal science and an increase in technology, the survival rates of infants with smaller gestational weeks have increased. In parallel with the increase in the survival rates of small premature babies, chronic diseases of premature infants are also increasing. As gestational week shrinks and survival rates increase, with its increased incidence, BPD is a serious chronic lung problem that still affects the lives of premature babies. In line with the literature, it was found that the incidence of BPD increased as gestational week and birth weight decreased (1). In the study of Rocha et al. (12), it was found that the SGA was an independent risk factor for BPD. In our study, the SGA baby ratio was higher in the BPD group (p=0.047).

In the literature, a case of an adult with agranulocytic eosinophilia due to the use of fluconazole has been reported (13). In the study, in which Yen and his colleagues (5) evaluated 142 preterm babies under 1500 grams, they did not determine the relationship between eosinophilia and fluconazole use. Similarly, in our study, there was no statistical difference between the BPD group and the non-BPD group in terms of the use of fluconazole (p=0.078). Other drugs that could increase eosinophil and used by the patients in our study during

the study were also examined. The number of patients using vancomycin and ibuprofen in the study group was significantly higher in the BPD group. While these drugs are likely to contribute to the increase in eosinophil count, we analyzed data from five different periods to minimize the effects and confounding factors of the drugs on study results. In the study of Yang and his colleagues (3), it was found that the incidence of eosinophilia increased in infants with sepsis among preterms younger than a 34-gestational week. In our study, the incidence of eosinophilia and sepsis was higher in the group with BPD, and the age of sepsis was earlier. Patel and his colleagues also found that sepsis and eosinophilia were associated with newborns (14). In the study of Jensen et al. (15) sepsis and SGA were defined as risk factors for BPD. Similarly, in our study, the incidence of eosinophilia and sepsis in the BPD group was higher, and the age of sepsis was earlier.

In our study, all patients in the study and control group received total parenteral nutrition (TPN). In the BPD group, TPN duration was longer than the control group (p=0.001). In the study group, with lower gestational week and birth weights, a longer TPN duration was an expected case. In our study, the need for intubation (at any time in the follow-up at birth and/or intensive care unit) and the need for blood trans-

fusion were also significantly higher in the study group compared to the control group. In the study group with lower gestational week and birth weights, a longer TPN duration, the need for intubation and more blood transfusions were the expected case. In the study of Bhat and his colleagues (16), and of Fayon and his colleagues (17), the relationship was determined between eosinophilia and TPN use, intubation, and blood transfusion.

While there is no statistical difference in leukocyte values at all times between the study group and the control group; eosinophil and basophil values were statistically higher in the BPD group than in the control group. This result shows that the number of eosinophils and basophils increased in patients with BPD without an increase in the number of leukocytes. Increased number of eosinophils and basophils suggests that cytokine response increases and consequently, inflammation damage and fibrosis in the lungs develops.

According to our information, studies in the literature have always investigated the relationship between severe eosinophilia and the frequency of BPD (3,5); in our study, unlike the literature, it has been shown that the numerical values of eosinophilia increase as the BPD grades increase. This result suggests a linear relationship between the number of eosinophils and the BPD grade, and that the increase in the number of eosinophils may be an indirect indicator of inflammation in the lungs and the associated damage. However, because the number of patients in the severe BPD group is small, this issue needs to be investigated and confirmed by studies using histochemical and biochemical markers, especially in infants with severe BPD.

Since the gestational week was lower in the study group, the frequency of PDA, IVH, NEC, and ROP was statistically higher than in the control group. Besides, in etiopathogenesis, if one of the diseases, where oxygen radical damage contributes occurs, the risk of other diseases' occurrence is increased. Likewise, the increase in the frequency of NEC and ROP along with BPD is due to the increase in the risk of underlying oxygen radical damage. However, there is no indirect or direct correlation shown between eosinophilia and/or basophilia and the severity of oxygen radical damage. Given that the increase in eosinophilia may be directly proportional to the severity of the inflammation response, oxygen radical damage resulting from the bactericidal activity may have contributed to BPD etiopathogenesis.

There was no difference between the groups with and without BPD in terms of the frequency of respiratory distress syndrome (RDS), the absence of this difference does not mean that there may be no statistical difference in terms of eosinophilia and basophilia. Today, in cases defined as "new BPD", BPD occurs without RDS (18).

Yamamoto et al. (6) in their study to investigate the pathol-

ogy of eosinophilia, examined the number of peripheral eosinophils, the core number of eosinophils, serum eosinophilic cationic protein levels, and polymorphonuclear leukocyte elastase levels in the first four weeks of life. They thought that in premature babies with BPD, peripheral eosinophils may be activated and appear to be related to the severity of BPD. Since our study was retrospective, only the peripheral eosinophil count was evaluated.

In the study group, compared to the control group, there was a statistically significant difference in eosinophil values and eosinophil severity in the first day, first week, second week, third week, and fourth week. Since the difference may be due to gestational week and birth weight, after remedial analysis on gestational week and birth weight was performed, it was observed that significant statistical differences continued to exist between the groups at all times ($p=0.001$). In the study of Yen and colleagues (5), eosinophilia was detected in the first week in the group with BPD for eight weeks.

In our study, eosinophil values were significantly higher at all times in the BPD group compared to the control group. In our study, the highest eosinophil values were reached in the first week. In the eight-week study of Yang et al. (3) the BPD group showed an increase in eosinophil values until the fourth week, followed by a decline in eosinophil values. In our study, eosinophil values were significantly higher in the study group during the four-week follow-up period, and the highest eosinophil values were recorded in the third week.

According to our knowledge, there is no study in the literature investigating the relationship between BPD and basophilia. Basophils typically enter circulation after maturation in the bone marrow and can infiltrate tissues of active inflammation areas in parasitic diseases, asthma, and allergies. Typically, the basophil has a relatively short life span ranging from a few days to several weeks. Various cytokines, especially Interleukin-3 (IL-3), stimulate the release of basophils from the bone marrow and prolong their lifespan (19,20). Besides, IL-3 increases eosinophil production (21). In our study, after remedial analysis on gestational week and birth weight was performed, basophilia values were also found to be statistically high in the BPD group during the period of eosinophilia. The parallel increase in eosinophil and basophil counts was thought to be an indirect indicator of cytokine response. Our study is compromised in the sense that inflammation markers and cytokine levels could not be investigated since it was a retrospective study. In our study, we found that eosinophil and basophil counts increased in the BPD group without an increase in the number of leukocytes. Therefore, there is a need for prospective clinical studies using biomarkers to investigate whether the increase in the eosinophil and basophil counts without an increase in the number of leukocytes is related to the increase in BPD risk and severity through an inflammatory response. Furthermore, it is neces-

sary to investigate whether steroid use in the treatment of BPD suppresses eosinophil-basophil count and inflammation response. In newborns, especially in premature infants, an increased number of eosinophils and/or basophils due to insufficient inflammation response and inflammation control resulting from an immature immune system is probably not related to the predisposition to allergies. However, studies should be conducted to investigate biomarkers with long-term follow-up results that can support this idea. There were very few individuals with allergic diseases in the study and control groups (5 and 1 case, respectively), which was thought to have no confounding effect on eosinophil and basophil counts. Today, the response to steroid therapy is only clinically evaluated. Conducting these studies can enable the response to the treatment to be evaluated with concrete markers.

Broström et al. (22) found that the response of eosinophils regressed after steroid treatment in their study where they evaluated the relationship between BPD and eosinophilia.

Various studies in the literature provide evidence that activation of eosinophils can contribute to BPD etiopathogenesis. According to the findings obtained in our study, we think that both eosinophil and basophil activation may have a role in the etiopathogenesis of BPD.

Prospective clinical studies including related biomarkers are needed to clarify the contribution of eosinophilia and basophilia in the etiology of bronchopulmonary dysplasia.

Conclusions

Our study shows that the number of eosinophils and basophils increased in patients with BPD without an increase in the number of leukocytes.

References

1. Gharehbaghi MM, Peirovifar A, Ghojazadeh M, Mahallei M. Efficacy of azithromycin for prevention of bronchopulmonary dysplasia. Turk J Med Sci. 2012;42(6): 1070-5. Doi: 10.3906/sag-1107-18.

2. Kalikkot Thekkeveedu R, Guaman MC, Shivanna B. Bronchopulmonary dysplasia: A review of pathogenesis and pathophysiology. Respir Med. 2017;132:170-7. Doi: 10.1016/j.rmed.2017.10.014.

3. Yang JY, Cha J, Shim SY, Cho SJ, Park EA. The relationship between eosinophilia and bronchopulmonary dysplasia in premature infants at less than 34 weeks' gestation. Korean J Pediatr. 2014;57(4):171-7. Doi: 10.3345/kjp.2014.57.4.171.

4. Juul SE, Haynes JW, McPherson RJ. Evaluation of eosinophilia in hospitalized preterm infants. J Perinatol. 2005;25(3):182-8. Doi: 10.1038/sj.jp.7211226.

5. Yen JM, Lin CH, Yang MM, Hou ST, Lin AH, Lin YJ. Eosinophilia in very low birth weight infants. Pediatr Neonatol. 2010;51(2):116-23. Doi: 10.1016/S1875-9572 (10)60021-6.

6. Yamamoto C, Kojima T, Hattori K, Nogi S, Imamura H, Tsubura A, et al. Eosinophilia in premature infants: correlation with chronic lung disease. Acta Paediatr. 1996;85 (10):1232-5. Doi: 10.1111/j.1651-2227.1996.tb18235.x.

7. Sticco KL, Lynch DT. Basophilia. 2019 Nov 7. StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2019 Jan. PMID: 30570986.

8. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163(7):1723-9. Doi: 10. 1164/ajrccm.163.7.2011060.

9. Tollner U. Early diagnosis of septicemia in the newborn. Clinical studies and sepsis score. Eur J Pediatr. 1982;138 (4):331-7. Doi: 10.1007/BF00442511.

10. Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med. 2005;6(3 Suppl):S45-9. Doi: 10.1097/01.PCC.0000161946.73305.0A.

11. Celik HT, Portakal O, Yigit S, Hascelik G, Korkmaz A, Yurdakok M. Efficacy of new leukocyte parameters versus serum C-reactive protein, procalcitonin, and interleukin-6 in the diagnosis of neonatal sepsis. Pediatr Int. 2016;58(2):119-25. Doi: 10.1111/ped.12754.

12. Rocha G, de Lima FF, Machado AP, Guimaraes H, Proença E, Carvalho C, et al. Hypertensive Disorders of Pregnancy Study Group. Small for gestational age very preterm infants present a higher risk of developing bronchopulmonary dysplasia. J Neonatal Perinatal Med. 2019; 12(4):419-27. Doi: 10.3233/NPM-180129.

13. Wong-Beringer A, Shriner K. Fluconazole-induced agranulocytosis with eosinophilia. Pharmacotherapy. 2000;20 (4):484-6. Doi: 10.1592/phco.20.5.484.35058.

14. Patel L, Garvey B, Arnon S, Roberts IA. Eosinophilia in newborn infants. Acta Paediatr. 1994;83(8):797-801. Doi: 10.1111/j.1651-2227.1994.tb13146.x.

15. Jensen EA, Schmidt B. Epidemiology of bronchopulmonary dysplasia. Birth Defects Res A Clin Mol Teratol.

2014;100(3):145-57. Doi: 10.1002/bdra.23235.

16. Bhat AM, Scanlon JW. The pattern of eosinophilia in premature infants. A prospective study in premature infants using the absolute eosinophil count. J Pediatr. 1981;98 (4):612. Doi: 10.1016/s0022-3476(81)80776-7.

17. Fayon M, Babin JP. Hypereosinophilia in premature newborn infants. Pediatrie. 1988;43(8):667-72. PMID: 3065720.

18. Voynow JA. "New" bronchopulmonary dysplasia and chronic lung disease. Paediatr Respir Rev. 2017;24:17-8. Doi: 10.1016/j.prrv.2017.06.006.

19. Mitre E, Nutman TB. Basophils, basophilia and helminth infections. Chem Immunol Allergy. 2006;90:141-56. Doi:10.1159/000088886.

20. Miura K, Saini SS, Gauvreau G, MacGlashan DW Jr. Differences in functional consequences and signal transduction induced by IL-3, IL-5, and nerve growth factor in human basophils. J Immunol. 2001;167(4):2282-91. Doi: 10.4049/ jimmunol.167.4.2282.

21. Bessler H, Straussberg R, Gurary N, Aloni D, Sirota L. Effect of dexamethasone on IL-2 and IL-3 production by mononuclear cells in neonates and adults. Arch Dis Child Fetal Neonatal Ed. 1996;75(3): F197-201. Doi:10.1136/ fn.75.3.f197.

22. Broström EB, Katz-Salamon M, Lundahl J, Halldén G, Winbladh B. Eosinophil activation in preterm infants with lung disease. Acta Paediatr. 2007;96(1):23-8. Doi: 10. 1111/j.1651-2227.2006.00002.x.