A Novel Diagnostic Marker for the Severity of Bronchopulmonary Dysplasia in Very Low Birth Weight Infants: Interleukin-33

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Background: Bronchopulmonary dysplasia (BPD) is an important clinical problem for premature infants. Previously, some biomarkers associated with severity of BPD have been studied. In our study, we aimed to investigate the value of interleukin-33 (IL-33) levels as a new biomarker in the follow-up of BPD severity and response to treatment.

Materials and Methods: Premature infants of <32 weeks of gestational age and birth weight <1,500 g were included in the study. Infants with BPD were divided into moderate and severe BPD groups. Infants without BPD were assigned as the control group. Cord blood samples were taken from both groups immediately after birth. In addition, blood samples were obtained at the time of diagnosis of BPD and after the end of hydrocortisone (HC) treatment to measure IL-33 values in the serum.

Results: During the study period, a total of 192 infants were eligible: 96 infants in the BPD group and 96 in the control group. Cord IL-33 values were similar between control (1.29 ± 0.68 pg/mL) and BPD (moderate/severe) (1.31 ± 0.84 pg/mL) groups ($P=0.813$). The levels of IL-33 were higher in BPD group (3.43 ± 0.98 pg/mL) than in the control group (0.98 ± 0.51 pg/mL) ($P<0.001$). IL-33 values decreased significantly after HC treatment (pretreatment: 3.43 ± 0.98 pg/mL versus post-treatment: 2.97 ± 0.28 pg/mL) ($P<0.001$). In addition, IL-33 levels were significantly higher in severe BPD (3.91 ± 1.22 pg/mL) than in moderate BPD (2.82 ± 0.74) group ($P<0.001$).

Conclusions: Although the IL-33 level was not predictive of the development of BPD immediately after birth, it may be used as a new biomarker to diagnose, monitor, and follow the response to treatment of BPD.

Keywords: bronchopulmonary dysplasia, hydrocortisone, interleukin-33, infant, very low birth weight

Introduction

BRONCHOPULMONARY DYSPLASIA (BPD), first described in 1967 by Northway et al., was defined as pulmonary disease due to mechanical ventilation in premature infants with respiratory distress syndrome (RDS).¹ Subsequently, the National Institute of Health consensus conference defined BPD as mild, moderate, or severe according to respiratory support at 28 days of age and 36 weeks of postmenstrual age (PMA).² The survival rate of preterms increase due to significant advances in respiratory care in the newborn. However, BPD is still an important clinical problem as the most common pulmonary morbidity.³ In addition to the genetic predisposition, it is argued that some biomarkers increase the susceptibility to BPD. Some cellular or humoral biomarkers contribute to the pathogenesis of BPD. These

biomarkers can be both mediators of pathophysiology and biomarkers of the disease.⁴

Interleukin-33 (IL-33) is a new cytokine of the IL-1 family found in 2005.⁵ IL-33 has been reported to be associated with the severity of many allergic and inflammatory diseases such as asthma, atopic dermatitis, autoimmune diseases, and chronic obstructive pulmonary disease (COPD).^{6–11} Although IL-33 has been shown to play an important role in various inflammatory and immunological pathologies, there is little information about its role in BPD. We therefore aimed to ascertain the association between the severity of BPD and serum IL-33 levels in very low birth weight (VLBW) preterm infants. In addition, the second aim of our study was to investigate the utility of IL-33 levels in evaluating response to hydrocortisone (HC) treatment in infants with BPD.

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Materials and Methods

Patient selection and study design

Our study was a prospective nested case-control study conducted in the neonatal intensive care unit (NICU) of Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey, from November 2015 to May 2018. The local ethical committee approved the study protocol. All parents signed the informed consent before the study. The NICU at our hospital was a referral Level III facility.

All infants with gestational age (GA) of <32 weeks and birth weight (BW) of <1,500 g were followed in NICU. Infants with congenital respiratory system anomalies, chromosomal anomalies, major congenital malformations, pneumonia, sepsis (at the time of serum sample collection), and mild BPD were excluded. Infants whose parents did not give permission were excluded from the study. VLBW infants with all moderate/severe BPD were treated with HC at PMA 36 weeks, and assigned as the study group. Premature infants who were fully fed and clinically stable, who did not receive respiratory support by invasive or noninvasive ventilation were allocated as the control group. At PMA 36 weeks, all VLBW infants were followed and treated according to the same NICU protocol.

Gender, BW, GA mode of delivery, Apgar score at first and fifth minutes, antenatal steroid administration, achievement of full enteral feeding, duration of noninvasive ventilation and mechanical ventilation, duration of oxygen support, RDS, patent ductus arteriosus (PDA), intraventricular hemorrhage (IVH; grade ≥ 3), necrotizing enterocolitis (NEC; grade ≥ 2), retinopathy of prematurity (ROP) requiring laser treatment, and length of NICU stay were recorded.

Infants with RDS were identified as requiring surfactant administration for any situation.¹² ROP was determined by skilled ophthalmologists based on the international classification of ROP.¹³ IVH was identified by cranial ultrasonography examination in the first 7 days of life.¹⁴ Bell's criteria were considered to identify NEC.¹⁵ According to consecutive echocardiographic examinations and clinical criteria, infants with PDA were treated with either certain drugs (ibuprofen and/or paracetamol)/or surgical ligation.¹⁶ The diagnosis of sepsis (clinic and culture proven) was done according to the previously described diagnostic criteria.¹⁷

Defining BPD

Infants with moderate or severe BPD were included in the study group. BPD was defined according to oxygen requirement or positive pressure ventilation therapy for a certain period (days) (e.g., at PMA 36 weeks or over postnatal day 28). Mild BPD was considered for infants who had oxygen requirement throughout the first 28 days but not at 36 weeks of PMA or at discharge. Moderate BPD was identified as oxygen requirements over 28 days of postnatal life plus oxygen supplementation with <30% oxygen level at 36 weeks of PMA. Severe BPD was defined as oxygen dependence with $\geq 30\%$ oxygen requirement over the first 28 days of postnatal life and/or positive pressure support at 36 weeks of PMA.¹⁸ One experienced neonatologist ensured the diagnosis of BPD. All the same examiners were blinded to HC treatment and IL-33 measurements.

HC treatment protocol for BPD

Eligible infants with moderate or severe BPD at PMA of 36 weeks were treated with HC and included in the study.³ Standard clinical dosage schemes for HC were fulfilled. HC with 5 mg/kg/day was separated into 4 different doses for 7 days, and a subsequent decreasing dose was administered for each 5 days. A total cumulative dosage of 72.5 mg/kg and a period of 22 days for a standard therapy were completed for every selected infant.¹⁹ Infants with moderate/severe BPD were evaluated with clinical examination and laboratory studies [X-ray, C-reactive protein (CRP), IL-6] to rule out infections such as pneumonia and sepsis before initiating HC therapy. Preterm infants with contraindications such as osteopenia (alkaline phosphatase levels >700 U/L), hyperglycemia (serum glucose levels >200 mg/dL), hypertension (systolic and diastolic blood pressure >95% percentile instituted based on age), and infections such as pneumonia, urinary tract infection, meningitis, sepsis, and cardiac hypertrophy identified by echocardiography were not treated with HC and were excluded from the study.

Blood sampling

Umbilical cord blood samples were obtained from infants with GA <32 weeks and BW <1,500 g. Subsequently, blood samples were obtained from infants who were diagnosed with moderate or severe BPD at 36 weeks of PMA. Also, blood samples for the measurement of IL-33 were obtained before starting and after HC treatment. The levels of IL-33 were evaluated in the control group at 36th week of PMA. Simple glass tubes covering no separation gel or preservatives were used for the collection of blood samples. Clotting of blood was waited for a while (30 min) at 4°C, and subsequently centrifuged at 3,000 rpm at 4°C for 10 min. One milliliter of serum aliquots were separated and frozen at -80°C until evaluation. Before starting measurement, serum samples with a trace amount of hemolysis were rejected and completely excluded from the study.

Biochemical analysis

IL-33 levels were analyzed by using a commercially available kit for IL-33 (sensitivity value = 0.2 pg/mL, Human IL-33 Platinum ELISA; eBioscience, Lot No.: 126699018) with enzyme-linked immunosorbent assays method (ELISA) (ELISA device, washer-reader Awareness Chromate model, Awareness Stax 2600 model; Awareness Technology, Inc., Palm City, FL). Results were stated as pg/mL for IL-33.

A commercially available CRP kit (IMAGE device, the Beckman Coulter, sensitive value = 0.8 mg/dL) was used to measure serum CRP levels by nephelometric method. And also, a commercially available Interleukin 6 (IL-6) kit (sensitivity value = 2 pg/mL, Siemens Health care Products Ltd.) was chosen to evaluate serum IL-6 levels through solid-phase enzyme-labeled chemiluminescent immunometric assay method (Immulite 2000 device).

Statistical analysis

Statistical Package for Social Sciences (SPSS) (version 15 for Windows; SPSS, Inc., St. Louis, MO) was applied for statistical analysis. *P* value of 0.05 was considered significant. Nonparametric continuous variables for independent

samples were analyzed by using Student's *t*-test and/or Mann–Whitney *U*-test, and categorical variables were analyzed by application of chi-square or Fisher's exact test. Findings were stated as median (minimum–maximum) and/or mean $\pm$ standard deviation for continuous variables. In addition, categorical variables and distribution of frequency were presented as percentage. Receiver operating characteristic (ROC) analysis and the area under the curve were applied to identify the diagnostic value of IL-33 levels in patients diagnosed with moderate and severe BPD.

Results

During the study period, 795 infants with VLBW and GA <32 weeks were evaluated. Six hundred three infants were excluded according to the exclusion criteria, and a total of 192 infants were included. The groups were assigned as the control ($n=96$) and BPD (moderate/severe) ($n=96$). A flow chart of the eligible infants is shown in Figure 1.

Mean GA and BW were 27.6 ± 1.8 weeks and 968 ± 191 g, respectively. BW, GA, and Apgar scores (at the first and fifth minutes) were lower in the “BPD” group ($P < 0.05$). The duration of noninvasive and invasive mechanical ventilation and oxygen support, the length of hospital stay were longer in the “BPD” group ($P < 0.05$). The length of hospital stay was longer in the “BPD” group ($P < 0.05$). There were no significant differences between the control and BPD groups in terms of gender, modes of delivery, antenatal steroid treatment, achievement of full enteral feeding time, and comorbidities of infants (PDA, IVH, and NEC) ($P > 0.05$). No significant differences were determined between the control and study groups in terms of serum CRP and IL-6 levels ($P = 0.647$, $P = 0.214$, respectively). A significant difference was shown between (moderate/severe) BPD and control groups in terms of IL-33 levels, RDS, and ROP ($P < 0.001$, $P = 0.021$, and $P = 0.011$, respectively) (Table 1). No significant differences were identified between control (1.29 ± 0.68 pg/mL) and BPD (moderate/severe) (1.31 ± 0.84 pg/mL) groups in terms of cord blood IL-33 levels ($P = 0.813$).

Infants in severe BPD group ($n=33$, 34.3%) had lower GA (25.1 ± 1.1 weeks versus 27.2 ± 1.5 weeks) and lower BW (741 ± 127 g versus 910 ± 176 g) compared with the infants in the moderate BPD group ($n=63$, 65.7%) ($P < 0.001$, $P = 0.001$, respectively). Moderate+severe BPD, only moderate and only severe BPD groups showed significant decrease in IL-33 levels after HC treatment ($P < 0.001$, $P = 0.001$, and $P = 0.014$, respectively). IL-33 levels were significantly higher in severe BPD group than in moderate group before and after HC treatment ($P < 0.001$, $P < 0.001$, respectively) (Table 2). After HC treatment, IL-33 levels in both severe (3.38 ± 0.34 pg/mL) and moderate BPD (2.08 ± 0.15 pg/mL) groups were still higher than those in control group (0.98 ± 0.51 pg/mL) ($P < 0.001$). It was determined that HC treatment decreased IL-33 levels.

ROC analysis was performed to determine the predictive diagnostic value of serum IL-33 levels in patients with moderate BPD. Area under the ROC curve was 0.833 (95% confidence interval = 0.693–0.926, $P = 0.0001$). The cutoff value of IL-33 was calculated as 1.88 with Youden's index, sensitivity 100% (95% confidence interval = 84.0–100.0), and specificity 100% (95% confidence interval = 86.7–100.0) (ROC curve is shown in Fig. 2A).

ROC analysis was implemented to identify the diagnostic value of IL-33 levels in patients diagnosed with severe BPD. Area under the ROC curve was found to be 0.857 (95% confidence interval = 0.724–0.989, significance level $P = 0.0001$). The IL-33 cutoff value was calculated to be 3.34 with the Youden index, sensitivity 95% (95% confidence interval = 75.1–99.2), and specificity 100% (95% confidence interval = 87.6–100.0) (Fig. 2B).

Discussion

To our knowledge, our study is the first study to investigate the relationship between the severity of BPD and IL-33 in premature infants. In our study, the IL-33 level was found to be higher in infants with BPD compared with the

FIG. 1. Showing the flow chart of the preterm infants in the study groups.

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE GROUPS

Demographic and clinical characteristics	Control group (n = 96)	BPD group (moderate/severe) (n = 96)	P
GA, weeks ^a	29.2 ± 1.9	26.1 ± 1.8	<0.001*
BW, g ^a	1,109 ± 228	825 ± 152	<0.001*
Gender, n (%)			
Male	47 (48.9)	49 (51)	0.813
Mode of delivery, n (%)			
Vaginal delivery	31 (32.3)	32 (33.3)	0.532
Apgar score first minute ^b	6 (2–8)	5 (1–7)	<0.001*
Apgar score fifth minute ^b	8 (4–9)	7 (3–8)	<0.001*
Antenatal steroid, n (%)	50 (52.1)	47 (48.9)	0.661
Comorbidities, n (%)			
RDS	57 (59)	74 (77.1)	0.021*
PDA	27 (28)	33 (34.3)	0.118
IVH	9 (9.4)	11 (11.4)	0.316
NEC	10 (10.4)	13 (13.5)	0.172
ROP	7 (7.2)	14 (14.5)	0.011*
MV duration, days ^a	4.1 ± 2.8	16.8 ± 13.4	<0.001*
NIV duration, days ^a	6.5 ± 3.9	25.5 ± 16.8	<0.001*
Oxygen duration, days ^a	11.5 ± 7.2	74.4 ± 26.6	<0.001*
Achievement of full enteral feeding, day ^a	10.1 ± 2.8	13.4 ± 4.5	0.098
Hospital stay, days ^a	54.3 ± 15.8	95.7 ± 32.7	<0.001*
CRP (mg/dL) ^a	2.1 ± 3.6	2.1 ± 3.8	0.647
IL-6 (pg/mL) ^a	13.3 ± 18.7	12.2 ± 23.5	0.214
IL-33 (pg/mL) ^a	0.98 ± 0.51	3.43 ± 0.98	<0.001*

*Statistically significant *P* values are highlighted.

^aMean ± standard deviation.

^bMedian (minimum–maximum).

BPD, bronchopulmonary dysplasia; BW, birth weight; CRP, C-reactive protein; GA, gestational age; IL-6, interleukin-6; IL-33, interleukin-33; IVH, intraventricular hemorrhage; MV, mechanical ventilation; NEC, necrotizing enterocolitis; NIV, noninvasive ventilation; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity.

control group, and the IL-33 level was positively correlated with the severity of BPD. It was also found that IL-33 levels decreased after HC treatment due to decreased BPD severity. However, cord blood IL-33 levels in the BPD and control groups were similar. IL-6 and CRP levels were measured in both groups to exclude inflammatory processes such as sepsis. IL-6 and CRP levels were normal and similar in both groups.

IL-33, a novel cytokine that is released from damaged and necrotic cells and possesses many functions, has been reported to induce T helper 2 immune response. IL-33 plays an important role in binding to its receptor ST2 (suppression

of tumorigenicity 2) through regulating inflammation and immunity.¹⁰ In addition, IL-33 is known to be structurally expressed in epithelial and endothelial cell barriers, some vascular beds, as well as lung airway epithelium.^{20,21} Recent studies have provided that not only airway epithelial cells but also hematopoietic cells such as macrophages and dendritic cells can produce IL-33.²²

IL-33 plays an important role in the immunity of mucosal tissues, inflammation, homeostasis, and in the pathogenesis of severe human airway diseases.²³ IL-33 has been declared to be associated with disease severity in allergic and pulmonary inflammation including asthma.^{6,9–11,24} Increased IL-33 levels have been suggested to be associated with airway and systemic inflammation in COPD, in addition to the pathogenesis and progression of COPD.⁷

Postnatal immune maturation and lung development in the process of BPD are not fully understood. Although the time interval is different, the structural properties of postnatal lung development are similar in humans and mice. Current studies have reported that the development of type 2 cells and the production of epithelial IL-33 during the alveolar period of mice are higher.⁸ In a recent experimental study, IL-33 has been shown to increase hyperoxia-induced BPD in neonatal mice by expression of inflammatory mediators. In contrast, IL-33 neutralizing antibodies have been shown to reduce BPD progression by inhibiting proinflammatory cytokines and apoptosis in lung tissue.²³

BPD is considered to be caused by the effects of fragile lung development and immature inflammatory process.

TABLE 2. INTERLEUKIN-33 LEVELS BEFORE AND AFTER HYDROCORTISONE TREATMENT IN BRONCHOPULMONARY DYSPLASIA GROUPS

Variables	Before HC treatment (IL-33 levels, pg/mL)	After HC treatment (IL-33 levels, pg/mL)	P
Moderate and severe BPD	3.43 ± 0.98	3.17 ± 0.28	<0.001*
Moderate BPD	2.82 ± 0.74	2.08 ± 0.15	0.001*
Severe BPD	3.91 ± 1.22	3.38 ± 0.34	0.014*
<i>P</i>	<0.001*	<0.001*	

*Statistically significant *P* values are highlighted.

BPD, bronchopulmonary dysplasia; HC, hydrocortisone; IL-33, interleukin-33.

FIG. 2. The ROC curve of interleukin-33, predicting (A) moderate and (B) severe bronchopulmonary dysplasia. ROC, receiver operating characteristic.

Many interleukins and cytokines that possibly contributed to this process have been studied, except IL-33.⁴ In this regard, our study was the first to evaluate the value of IL-33 in BPD. It was found that IL-33 levels were higher in BPD group compared with control group and in severe BPD group compared with moderate BPD group. Based on the data from experimental studies on IL-33, the production of IL-33 in both epithelial and hematopoietic cells in the lung explains its role in the development of an intensive inflammatory process in BPD.^{21–23}

In addition, previous studies have examined the effects of steroid treatment on IL-33 levels as well as the effects of IL-33 on infiltration of M2 macrophages and dendritic cells. As a result, steroid therapy has been shown to affect the infiltration of inflammatory cells producing IL-33, thereby reducing IL-33 levels.²² Steroids such as HC, which are frequently used in severe and moderate BPD treatment, reduce the incidence and severity of BPD due to their anti-inflammatory properties.^{19,25} According to our results, decrease in the severity of BPD and decrease of IL-33 levels by HC treatment suggest that IL-33 may contribute to the process of BPD. Furthermore, IL-33 levels in cord blood were found to be similar between groups. This result suggested that BPD developed due to many postnatal factors, and IL-33 levels increased during the development of BPD might contribute to the pathogenesis of BPD.²⁶

In addition to the traditional diagnostic methods of BPD, biomarkers can help diagnose, quantify the severity of the disease, and monitor response to the treatment. It may also help determine prognosis and establish additional therapies.⁴ Except for neonatal sepsis, the association between IL-33 and neonatal diseases has not been fully elucidated.²⁷ In this respect, IL-33 can be considered as a new biomarker that can be used to determine the severity of BPD, to monitor disease, and to monitor response to treatment.

Increased biomarkers (IL-1 β , -6, -8, -10, interferon- γ , granulocyte colony stimulating factor, endostatin, vascular endothelial growth factor, platelet-derived growth factor BB, placental growth factor, E selectin, and B-type natriuretic

peptide) in serum as well as some markers (IL-1 β , -6, -8, tumor necrosis factor- α , monocyte chemoattractant protein, nuclear factor kappa β , neutrophil gelatinase-associated lipocalin, MMP-9, transforming growth factor- β 1, L-selectin, Ang-2, endothelin-1, fibroblast growth factor-2, and pepsin) in tracheal aspirate were found to be related to the development and severity of BPD. However, according to our knowledge, all of these markers used were biomarkers evaluated immediately after birth or on the follow-up.⁴ An experimental study aimed to investigate the role of IL-33 in the lungs of neonatal mice with hyperoxia induced BPD has reported that IL-33 is related to BPD. Furthermore, a recent study declared that treatment with anti-IL-33 has caused a decrease in both severity of BPD and IL-33 levels.²³ There existed lack of studies evaluating biomarker levels obtained at birth, before/after BPD treatment, and associated with BPD severity. Therefore, this study was the first aimed to figure out that point.

There were some limitations in our study. It is clear that there are many pathophysiological mechanisms contributing to the development of BPD. Therefore, the similarity of IL-33 levels in both groups in cord blood showed that IL-33 could not be used in predicting which patient would have BPD. However, postnatal inflammatory process contributes significantly to the development of BPD. The results of our study showed that IL-33 that contributes to the inflammatory process might be effective in the development of BPD together with other inflammatory markers.

In conclusion, our study found that IL-33 levels in cord blood were similar in groups with and without BPD. This result supports the role of postnatal proinflammatory and devastating multiple risk factors in the development of BPD. In addition, IL-33 levels correlated with the severity of BPD, and anti-inflammatory treatments such as HC decreased both BPD severity and IL-33 levels in relation to decreasing BPD intensity. Eventually, IL-33 may be suggested as a promising new biomarker for BPD diagnosis, follow-up, and monitoring treatment response. However, further studies are recommended before any clinical application.

Acknowledgments

The authors thank all the nursing staff of neonatal intensive care unit in Zekai Tahir Burak Maternity Teaching Hospital. This study was supported by grants from Zekai Tahir Burak hospital education planning and coordination committee (grant number: 62/2016)

Author Disclosure Statement

No competing financial interests exist.

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Received for publication January 8, 2019; accepted after revision February 10, 2019.