The Cytokines, Exhaled Nitric Oxide are Risk Factors in Preterm Infants for Bronchopulmonary Dysplasia

Zhenjie Zhang

Peking Union Medical College Hospital

Wuchen Wu

Department of Neurosurgery, Shenzhen University General Hospital, Shenzhen, China

Lian Hou

Department of clinical lab, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

Jingjing Jiang

Department of Pediatrics, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

Yunwei Li

Department of Pediatrics, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

Weilin Wan (✉ wanweilin@yahoo.com)

Department of Pediatrics, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

Zhenghong Li

Department of Pediatrics, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

Research

Keywords: Bronchopulmonary dysplasia, cytokines, FeNO, Risk Factors

Posted Date: April 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-23901/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Bronchopulmonary dysplasia (BPD) remains the most frequently complication of extreme preterm infants. Multiple clinical factors and inflammatory markers have all been associated with BPD. Therefore, this study targeted to detect cytokines and fractional exhaled nitric oxide (FeNO) to evaluate their mechanism and possible predicted significance for BPD.

Methods

Preterm infants born at gestational age ≤ 32 weeks were recruited between January 2018 and October 2019. The clinical data of infant characteristics and maternal characteristics were collected. Our study detected a total of ten cytokines include IFN- γ , IL-10, IL-12p70, IL-13, IL-1 β , IL-2, IL-4, IL-6, IL-8 and TNF- α on day 1–3, day 7–14, and day 21–28 after birth via Meso Scale Discovery (MSD) technology. FeNO levels were measured when the infants met discharge criteria.

Results

A total of 46 preterm infants were enrolled in this study, including 14 infants in BPD group and 32 infants in control group. The gestational age (27.5 ± 1.3) vs. (29.9 ± 1.3) weeks and birth weight (1021 ± 261) g vs. (1489 ± 357) g of BPD group were lower than those of control group. Multivariate logistic regression analysis showed that gestational age < 30 weeks, birth weight < 1000 g, PDA, longer mechanical ventilation and invasive ventilation duration were high risk factors for BPD. The cytokines of IL-6, IL-8 on day 7–14 and IL-4, IL-6, IL-8, TNF- α on day 21–28 were also the high risk factors for BPD. Other risk factors for BPD included elevated Eosnophils on day 21–28 and FeNO.

Conclusion

The preterm infants with PDA, prolonged mechanical ventilation tended to develop BPD. The FeNO, Eosnophils, cytokines such as IL-4, IL-6, IL-8, TNF- α were high risk factors for BPD. Our study speculate that NO was related to BPD though Th2-cell-mediated inflammatory responses such as IL-4. The cytokines may provide a certain predictive value for the occurrence of BPD.

Background

Bronchopulmonary dysplasia (BPD) remains the most frequently complication of extreme preterm birth. Currently, it's recognized as the results of an aberrant respiratory distress response to the developing lungs. BPD is a major form of chronic lung diseases in preterm infants and is primarily due to respiratory distress syndrome (RDS). Arguably, mechanical ventilation and long-term use of oxygen may contribute

to BPD onset[1]. However, the incidence of BPD has not decreased as multiple strategies to provide ventilation and oxygen therapy. Although great advances have been made in animal BPD models, clinical research to explore the pathogenesis and treatment of BPD is imperative.

In preterm infants, episodes of sepsis and the ongoing use of supplemental oxygen, mechanical ventilation, and parenteral nutrition have all been associated with systemic inflammatory responses. Especially, it is associated with the effects of inflammatory cytokines and chemokines. In the tracheal aspirate samples, IL-6, IL-8, IL-10, and TNF- α are significantly altered in BPD infants[2]. In the present study, we advanced our research on inflammation and explored the risk conferred by selected inflammatory cytokines (IFN- γ , IL-10, IL-12p70, IL-13, IL-1 β , IL-2, IL-4, IL-6, IL-8, TNF- α) in venous blood of preterm infants.

Fractional exhaled nitric oxide (FeNO) is one of the most promising biomarkers for airway inflammation, and there is a good relationship between FeNO levels and eosinophilic airway inflammation. In 1993, a study was conducted to detect airway inflammation by measuring FeNO levels, and it was believed that it is significantly positively related to the severity of inflammation[3]. In 2009, the American Thoracic Society / European Respiratory Society (ATS / ERS) joint statement issued affirmed FeNO Clinical application as a biological indicator of airway inflammation[4]. In 2011, ATS published clinical guidelines for FeNO[5]. FeNO testing is simple, reliable, and reproducible, making it an ideal method for non-invasive evaluation of airway inflammatory diseases. Therefore, this study targeted preterm infants to detect cytokines and FeNO to evaluate their mechanism and possible predicted significance for BPD.

Methods

We conducted a prospective study, and the study was approved by the Ethics Institutional Review Board of Peking Union Medical College Hospital. The informed consents were signed when infants were admitted to the NICU if parents agreed to use the residual serum from routine testing for detecting the cytokines.

1.Study population

Inclusion criteria: (1)Preterm infants born at gestational age ≤ 32 weeks;(2)Preterm infants born From January 2018 to October 2019;(3)Preterm infants were admitted to the Neonatal Intensive Care Unit in Peking Union Medical College Hospital within three days after birth;(4) Parents signed the informed consents.

Exclusion criteria: (1)Infants with severe congenital malformations;(2)Infants with severe infection, shock;(3)Infants with inherited metabolic diseases;(4)Infants died, discharged or transported to other hospitals before they met discharge criteria.

Infants diagnosed with BPD were assigned to BPD group, and infants without BPD were assigned to control group. BPD was diagnosed as oxygen dependency >28 days after birth, according to the

workshop definition by the National Institutes of Child Health, the Human Development/National Heart, Lung, and Blood Institute and the Office of Rare Diseases[6].

2. Data collection and Analysis

Clinical data were prospectively collected by a single investigator and verified by another. The baseline data of infant characteristics were collected, including gestational age, birth weight, Apgar score, Neonatal Respiratory Distress Syndrome(NRDS), anemia, patent ductus arteriosus(PDA), eosinophils, duration of mechanical Ventilation(days), duration of invasive ventilation(days),et al. Eosinophilia was defined as an absolute eosinophil count more than $700/\text{mm}^3$ [7]. The baseline data of maternal characteristics were collected, including delivery mode, antenatal steroids, premature rupture of membrane(PROM),et al.

3. Inflammation Biomarkers

To avoid a sample selection bias, parents or legal guardians of eligible infants were approached as early as possible after birth (usually <72 hours). Infants were sampled blood between birth and 30 days of age according to the clinical needs for routine clinical testing weekly. To limit invasiveness, analyses for the research were carried out only from residual (leftover) serum of routine testing from the hospital laboratory. So the inflammation biomarkers were tested once a week. The cytokines of serum were detected on day 1-3, day 7-14, and day 21-28 after birth. Our study detected a total of ten cytokines include IFN- γ , IL-10, IL-12p70, IL-13, IL-1 β , IL-2, IL-4, IL-6, IL-8 and TNF- α . To minimize protein degradation, our hospital laboratory employed strict protocols where blood collected in BDMicrotainers (Becton Dickinson, Mississauga, Ontario, Canada) was spun down within 1 hour to remove the cell fraction, and leftover serum was immediately stored at -80°C . The serum was collected for the cytokine protein level detection via Meso Scale Discovery (MSD) technology using a U-PLEX Biomarker Group 1 (Human) Multiplex Assay (K15049D). A MESO QuickPlex SQ 120 machine and MSD Discovery Workbench software version 4.0.12 were used for the detection and data analyses, respectively.

4. Detection of FeNO levels

FeNO levels were measured when the infants met discharge criteria (weight>2000g, Corrected gestational age>34w, Fulloral feeding). No bronchodilators or nebulized inhaled hormones were used 12 h before the test. The study subject was in a quiet sleep state, lying on his back, with a cushion height of about 2 cm under his or her shoulders, taking the nasal inhalation position, covering nose and mouth with a mask, tightening the mask along the breath. The nitric oxide filter guaranteed the inhalation of nitric oxide gas without impurities. FeNO was measured using the NIOX Flex (Aerocrine, Sweden), based on the principle of ozone-NO₂-based chemiluminescence. Calibrations were performed in accordance to manufacturer instructions. Before FeNO samples were obtained, samples of ambient NO in the room were collected. When ambient NO was below 5 parts per billion (ppb), measurements were conducted. Adequate ventilation of the room was assured. Analyses were conducted immediately after collection.

5. Statistics

Statistical analysis was performed using SPSS 25.0. The Quantitative data of normal distribution was described by the mean $\pm$ standard error of the mean(). The data between two groups were compared using the *t*-test. The Quantitative data that did not meet the normal distribution was described by M(P25-P75), and the comparison between groups was tested by rank sum test. The qualitative data was described by frequency and rate, the Pearson chi-square test or fisher's precise test was performed. Logistic regression model was used to analyze the independent risk factors of BPD. $P < 0.05$ was considered statistically significant.

Results

1. Baseline Data

From January 2018 to October 2019, 52 preterm infants were born at gestational age ≤ 32 weeks. Four preterm infants were excluded due to insufficient residual serum of routine testing for cytokines detect. Two preterm infants were excluded due to death from severe infection. A total of 46 preterm infants were enrolled in this study, including 14 infants in BPD group and 32 infants in control group. A total of 138 blood samples were collected. The incidence of BPD was 30% in our study. The gestational age (27.5 ± 1.3) vs. (29.9 ± 1.3) weeks $P = 0.000$ and birth weight (1021 ± 261) g vs. (1489 ± 357) g $P = 0.000$ of BPD group were lower than those of control group. As shown in Table 1-1 to Table 1-3. The incidence of PDA, anemia and NRDS was higher in BPD group, duration with mechanical ventilation, invasive ventilation, parenteral nutrition, length of hospital stay, however, were significantly longer in BPD group.

2. Inflammatory Cytokine Profile of the BPD Patients

The number of cytokine data points over time confirmed similar distribution between infants with or without BPD in the first 4 weeks. A total of 1380 times of cytokines were detected. The results of ten cytokines include IFN- γ , IL-10, IL-12p70, IL-13, IL-1 β , IL-2, IL-4, IL-6, IL-8 and TNF- α showed in Table 2. On day 1-3, IL-10 was significantly decreased in the BPD group. On day 7-14, IL-6, IL-8 and TNF- α were significantly increased in the BPD group while the IL-10 was decreased. On day 21-28, IL-1 β , IL-4, IL-6, IL-8 and TNF- α were significantly increased in the BPD group while the IL-10 was decreased. The comparison of cytokines between BPD and Non-BPD group was showed in the Figure 1-6.

3. Comparison of FeNO and Eosnophils between two groups

All the enrolled preterm infants were tested for FeNO before they discharged, there was no significant difference between two groups about the corrected gestational age on the test day $[(36.4 \pm 3.2)$ and (35.2 ± 2.3) weeks, $t = 1.407$, $P = 0.169$]. The FeNO of BPD group was higher than the control group $[(13.9 \pm 6.2)$ and (8.2 ± 3.3) ppb, $t = 2.967$, $P = 0.006$], and the differences were statistically significant (Table 1-3). The eosinophilia in the infants of the BPD group was significantly increased at 21-28 days after birth. The differences were statistically significant (P values < 0.05).

4. Multivariate logistic regression of BPD influencing factors

Multivariate logistic regression analysis was conducted in this study. The statistically significant factors in the univariate analysis were used as independent variables and BPD as the dependent variables (Table 3). The data showed that gestational age < 30 weeks, birth weight < 1000g, PDA, longer mechanical ventilation and invasive ventilation duration were high risk factors for BPD. The cytokines of IL-6, IL-8 on day 7-14 and IL-4, IL-6, IL-8, TNF- α on day 21-28 were also the high risk factors for BPD. Other risk factors for BPD included elevated eosinophils on day 21-28 and FeNO.

Discussion

In recent years, as the survival rate of Extremely Low Birth Weight Infant (ELBWI) and Very Low Birth Weight Infant (VLBWI) had increased, the incidence of BPD had increased rapidly. ELBWI / VLBWI was extremely immature due to the structure and function of the lung. Various damage factors, such as oxides, free radicals, hypoxia, infection, ventilator pressure, volume damage, or alveolar shear stress, etc., directly cause the release of inflammatory factors through pro-inflammatory factors, chemokines and proteases, and lead to BPD. BPD is a multifarious disease, including genetic background, maternal complications, and clinical syndromes caused by various clinical events in early life.

The gestational age and birth weight are the most important factors affecting the occurrence of BPD. The incidence of BPD in preterm infants with a gestational age of 30 weeks is 12%; the incidence with a gestational age of 25 to 26 weeks is 30–40% [8]. In this study, the gestational age and birth weight of the BPD group were low, and multivariate analysis showed that birth gestational age < 30 weeks and birth weight < 1000 g were risk factors for BPD. This was consistent to the previous research results. Other known risk factors for BPD included maternal preeclampsia [9], chorioamnionitis [10], sepsis [11] and fetal growth restriction [12], and early mechanical ventilation for > 7 days [13]. In our study, prolonged mechanical ventilation and invasive ventilation duration were risk factors for BPD, this was consistent to the previous research results. Recent meta-analysis suggested that strategies to avoid endotracheal ventilation had significant effects in preventing BPD [14].

In this study, although the proportion of patients with NRDS in BPD group was higher than that in non-BPD group, but multivariate analysis did not suggest that respiratory distress syndrome was a risk factor for BPD. It was considered to be related to the fact that the research subjects were all premature babies, most of them received prenatal corticosteroid and the pulmonary surfactant, prenatal corticosteroid and surfactant treatment in the BPD group improved the respiratory symptoms quickly.

Our study found that PDA was a high risk factor for BPD. We thought the reason was that PDA could prolong the duration of ventilator-assisted ventilation after birth. Severe left-to-right shunts in PDA infants could cause pulmonary congestion and edema. Long-term left-to-right shunts could cause increased pulmonary vascular damage and aggravated pulmonary inflammation. So, Minimizing the duration of mechanical ventilation as much as possible, actively closing the arterial catheter, performing

comprehensive treatment and standardized management may help reduce the incidence and risk of BPD to improve the prognosis of preterm infants.

Both prenatal and postnatal inflammation could cause imbalances of pro-inflammatory and anti-inflammatory factors in the body. A large number of pro-inflammatory factors were released, exacerbating immature lung damage and causing BPD. Previous animal experiments validated this result. In the previous study, IL-6 had been directly implicated in a murine hyperoxia model of BPD[15]. The results from clinical trials were consistent with this. In the BPD infants, the inflammatory cytokines IL-1 β , IL-6, TNF- α , and IL-10 levels were altered in the amniotic fluid[16], cord blood[17], and tracheal aspirate samples[18]. TNF- α single nucleotide polymorphisms could predict BPD onset and severity in preterm neonates[19]. Harald Ehrhardt et al. reported that TNF- α in tracheal aspirate samples are associated with BPD severity [20]. Therefore, we speculated that these cytokines may play a direct role in the lung injury that led to BPD in premature infants. In our study, we found that in the BPD preterm newborns, IL-6, IL-8 and TNF- α were significantly increased on days 7–14, and IL-1 β , IL-4, IL-6, IL-8, TNF- α were significantly increased on days 21–28. The anti-inflammatory cytokine IL-10, however, was significantly reduced in preterm patients with BPD on days 1–3, days 7–14, and days 21–28. The cytokines of IL-6, IL-8 at days 7–14 and IL-4, IL-6, IL-8, TNF- α at days 21–28 were the high risk factors for BPD. Our data had potential clinical implications. Our results reflect the dynamic change of cytokines at different time points. Some cytokines, such as IL-1 β , IL-4, IL-6, IL-8, TNF- α , showed no significant changes in the early stage and significant changes in the later stage. The cytokines may provide a certain predictive value for the occurrence of BPD. The observation of sustained systemic inflammation in infants prior to clinical symptoms of BPD suggested that anti-inflammatory interventions during early period may be more effective.

Because lung inflammation could impede lung maturation, FeNO is elevated due to ventilator-assisted breathing and inhaled oxygen. FeNO was a marker of airway eosinophil inflammation in adults and older children[21]. In infants with BPD, bronchial hyperresponsiveness may appear later in life, which was related to tracheal intubation and ventilator-assisted breathing in early life. May C had shown that exhaled nitric oxide levels were raised in infants with established BPD, particularly in those developing moderate or severe BPD, and may reflect ongoing inflammation[22]. Our study found that the FeNO and eosinophils of BPD group were higher than that of non-BPD group. The multivariate analysis showed that FeNO and elevated eosinophils on Day 21–28 were high risk factors for BPD. All these results showed that BPD was closely related to eosinophilic airway inflammation.

NO is generated by NO synthase (NOS). NO is involved in the Th2-cell-mediated (mainly eosinophils) inflammatory responses and has multiple roles from proinflammatory to anti-inflammatory actions, regulating bronchomotor tone. There are three NOS isoforms, namely endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). The relationship between NO and IL-4 is mutually affecting and promoting. To elucidate the authentic role of NO/NOSs, a mice model lacking all NOSs (n/i/eNOS-/-) was used in the previous research. Pathological findings of the n/i/eNOS-/- mice revealed a significant decrease in bronchial eosinophilic inflammation, bronchial thickening, mucus secretion and mRNA levels

of IL-4, IL-5, IL-13. It indicated that NO played an important role in promoting eosinophilic inflammation, mucus hypersecretion that might be related to an increase of Th2 cytokines such as IL-4, IL-5, IL-13[23]. On the contrary, IL-4 could also increase the expression of iNOS[24]. IL-4 is known to induce the alternative activation pathway in M2 macrophages, have been shown to regulate NO production by inducing arginase I, which catalyzes the hydrolysis of L-arginine, a common substrate of iNOS, and thereby regulates NO synthesis by competing with the substrate[25]. Expression of iNOS can be observed in epithelial, endothelial, neural, and smooth muscle cells, eosinophils, neutrophils, and alveolar macrophages in the lung after its induction by IL-4, and/or IL-13[26][27]. In our study, we found that both IL-4 and FeNO were increased and related to BPD by univariate analysis and multifactor analysis. We speculate that NO was related to BPD though Th2-cell-mediated inflammatory responses such as IL-4. However, excessive NO often induces immune and pathological processes and mediates the production of some inflammatory mediators, resulting in tissue damage[28].

One limitation of our study is that serum cytokine concentrations may not accurately reflect concentrations of the cytokines in the lung, and it is not possible to identify target cells in the lung which produced or responded to the cytokines. However, such limitation also existed with tracheal aspirates or bronchoalveolar lavage, which reflect the epithelial lining fluid rather than the alveolar or interstitial milieu. Additional studies are required to determine the relationship between changes in serum cytokines and concomitant lung pathology. It is also possible that changes in some of the serum cytokines reflect the magnitude of lung injury, with more severe lung injury resulting in greater cytokine concentrations. Some cytokine responses maybe attempt to compensate or attenuate the lung injury. Small sample size is another limitation of our study. We could not further assign BPD infants to different groups according their severity. Further studies with larger sample size are required to assess the values of cytokines and FeNO in the diagnosis of BPD.

Conclusion

In summary, BPD is a disease caused by a combination of multi-factors. It is closely related to eosinophilic airway inflammation. The preterm infants with PDA, prolonged mechanical ventilation tended to develop BPD. The FeNO, Eosnophils, cytokines such as IL-4, IL-6, IL-8, TNF- α are high risk factors for BPD. The cytokines may provide a certain predictive value for the occurrence of BPD. Our study speculated that NO was related to BPD though Th2-cell-mediated inflammatory responses such as IL-4. The observation of sustained systemic inflammation in infants prior to clinical symptoms of BPD suggested that anti-inflammatory interventions during early period may be more effective. We provide valuable insights that may be used to design intervention strategies targeting inflammation in preterm infants at risk of BPD.

List Of Abbreviations

Bronchopulmonary dysplasia (BPD)

fractional exhaled nitric oxide(FeNO)

Neonatal Respiratory Distress Syndrome(NRDS)

patent ductus arteriosus(PDA)

Declarations

- **Ethics approval and consent to participate**

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences. The ethical review number was ZS-1552.

We have obtained written informed consent from all study participants of parents. All of the procedures were performed in accordance with the Declaration of Helsinki and relevant policies in China.

- **Consent for publication**

Not applicable

- **Competing interests**

The authors disclose no conflicts.

- **Funding**

This work was supported by the CAMS Innovation Fund for Medical Sciences[CIFMS], NO. 2017-I2M-3-017.

- **Authors' contributions**

Zhenjie Zhang (Formal analysis; Methodology; Writing– original draft)

Wuchen Wu (Data curation)

Lian Hou (Investigation; Resources)

Jingjing Jiang (Data curation; Investigation)

Yunwei Li (Software; Supervision)

Weilin Wan (Funding acquisition; Methodology; Project administration)

Zhenghong Li (Writing – review & editing)

- **Acknowledgements**

The authors will thank all the patients for great help in the artical.

- **Availability of data and materials**

All data generated or analysed during this study are included in this published article

References

- [1]. Lal, C.V. and N. Ambalavanan, Cellular and humoral biomarkers of Bronchopulmonary Dysplasia. *Early Human Development*, 2017. 105: p. 35-39.
- [2]. Yilmaz, C., et al., Low serum IGF-1 and increased cytokine levels in tracheal aspirate samples are associated with bronchopulmonary dysplasia. *Turk J Pediatr*, 2017. 59(2): p. 122-129.
- [3]. Alving, K., E. Weitzberg and J.M. Lundberg, Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J*, 1993. 6(9): p. 1368-70.
- [4]. Reddel, H.K., et al., An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med*, 2009. 180(1): p. 59-99.
- [5]. Dweik, R.A., et al., An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med*, 2011. 184(5): p. 602-15.
- [6]. Jobe, A.H. and E. Bancalari, Bronchopulmonary dysplasia. *Am J Respir Crit Care Med*, 2001. 163(7): p. 1723-9.
- [7]. Gibson, E.L., Y. Vaucher and J.J. Corrigan, Eosinophilia in premature infants: relationship to weight gain. *J Pediatr*, 1979. 95(1): p. 99-101.
- [8]. Capasso, L., et al., Oxidative Stress and Bronchopulmonary Dysplasia: Evidences From Microbiomics, Metabolomics, and Proteomics. *Frontiers in Pediatrics*, 2019. 7: p. 30_x000a_.
- [9]. Eriksson, L., et al., Prenatal inflammatory risk factors for development of bronchopulmonary dysplasia. *Pediatr Pulmonol*, 2014. 49(7): p. 665-72.
- [10]. Lee, H.J., et al., Chorioamnionitis, respiratory distress syndrome and bronchopulmonary dysplasia in extremely low birth weight infants. *J Perinatol*, 2011. 31(3): p. 166-70.
- [11]. Klinger, G., et al., Outcome of early-onset sepsis in a national cohort of very low birth weight infants. *Pediatrics*, 2010. 125(4): p. e736-40.

- [12]. Shima, Y., S. Kumasaka and M. Migita, Perinatal risk factors for adverse long-term pulmonary outcome in premature infants: comparison of different definitions of bronchopulmonary dysplasia/chronic lung disease. *Pediatr Int*, 2013. 55(5): p. 578-81.
- [13]. Nobile, S., et al., New Insights on Early Patterns of Respiratory Disease among Extremely Low Gestational Age Newborns. *Neonatology*, 2017. 112(1): p. 53-59.
- [14]. Fischer, H.S. and C. Buhrer, Avoiding endotracheal ventilation to prevent bronchopulmonary dysplasia: a meta-analysis. *Pediatrics*, 2013. 132(5): p. e1351-60.
- [15]. Choo-Wing, R., et al., Developmental differences in the responses of IL-6 and IL-13 transgenic mice exposed to hyperoxia. *Am J Physiol Lung Cell Mol Physiol*, 2007. 293(1): p. L142-50.
- [16]. Yoon, B.H., et al., Amniotic fluid cytokines (interleukin-6, tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-8) and the risk for the development of bronchopulmonary dysplasia. *Am J Obstet Gynecol*, 1997. 177(4): p. 825-30.
- [17]. Rocha, G., et al., Cord blood levels of IL-6, IL-8 and IL-10 may be early predictors of bronchopulmonary dysplasia in preterm newborns small for gestational age. *Dis Markers*, 2012. 33(1): p. 51-60.
- [18]. Yilmaz, C., et al., Low serum IGF-1 and increased cytokine levels in tracheal aspirate samples are associated with bronchopulmonary dysplasia. *Turk J Pediatr*, 2017. 59(2): p. 122-129.
- [19]. Szpecht, D., et al., The significance of IL-1beta +3953C>T, IL-6 -174G>C and -596G>A, TNF-alpha -308G>A gene polymorphisms and 86 bp variable number tandem repeat polymorphism of IL-1RN in bronchopulmonary dysplasia in infants born before 32 weeks of gestation. *Cent Eur J Immunol*, 2017. 42(3): p. 287-293.
- [20]. Ehrhardt, H., et al., Absence of TNF-alpha enhances inflammatory response in the newborn lung undergoing mechanical ventilation. *Am J Physiol Lung Cell Mol Physiol*, 2016. 310(10): p. L909-18.
- [21]. Gabriele, C., et al., Exhaled nitric oxide and the risk of wheezing in infancy: the Generation R Study. *Eur Respir J*, 2012. 39(3): p. 567-72.
- [22]. May, C., et al., Relation of exhaled nitric oxide levels to development of bronchopulmonary dysplasia. *Arch Dis Child Fetal Neonatal Ed*, 2009. 94(3): p. F205-9.
- [23]. Akata, K., et al., Decreased Bronchial Eosinophilic Inflammation and Mucus Hypersecretion in Asthmatic Mice Lacking All Nitric Oxide Synthase Isoforms. *Lung*, 2016. 194(1): p. 121-124.
- [24]. Huang, H., et al., IL-4 stimulates the expression of CXCL-8, E-selectin, VEGF, and inducible nitric oxide synthase mRNA by equine pulmonary artery endothelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 2007. 292(5): p. L1147-L1154.

- [25]. Munder, M., et al., Th1/Th2-regulated expression of arginase isoforms in murine macrophages and dendritic cells. J Immunol, 1999. 163(7): p. 3771-7.
- [26]. Chibana, K., et al., IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. Clinical & Experimental Allergy, 2008. 38(6): p. 936-946.
- [27]. Yatera, K. and H. Mukae, Possible pathogenic roles of nitric oxide in asthma. Respiratory Investigation, 2019. 57(4): p. 295-297.
- [28]. Fan, W., et al., Relationship of T lymphocytes, cytokines, immunoglobulin E and nitric oxide with otitis media with effusion in children and their clinical significances. Revista da Associacao Medica Brasileira, 2019. 65(7): p. 971-976.

Tables

Table 1-1 The Basic Infant Characteristics Between BPD and Non-BPD Group

	BPD group(n=14)	Non-BPD group(n=32)	P-value
Male gender n(%)	9(64.3%)	16(50%)	0.370
Singleton n(%)	10(71.4%)	28(87.5%)	0.183
Birth weight (grams)	1021±261	1489±357	0.000
Gestational age (weeks)	27.5±1.3	29.9±1.3	0.000
Small Gestational Age (SGA) n(%)	0	1(3.1%)	1.000
Apgar 1min	7.5±2.5	8.4±1.3	0.140
Apgar 5min	8.9±1.5	9.5±0.8	0.090
Apgar 10min	9.3±0.9	9.6±0.6	0.084
NRDS n(%)	14(100%)	23(71.9%)	0.048
Patent ductus arteriosus (PDA) n(%)	12(85.7%)	12(37.5%)	0.004
Late-onset neonatal sepsis (LOS) n(%)	7(50%)	15(46.9%)	0.844
Anemia n(%)	14(100%)	23(71.9%)	0.030
Minimum HGB	101±8.7	105±12.9	0.220
Duration of mechanical ventilation (days) M(P25-P75)	36(27-53)	10(7-15)	0.000
Duration of invasive ventilation(days) M(P25-P75)	4(2.7-7.2)	0	0.000
Nebulized inhaled hormone and bronchodilator n(%)	8(57.1%)	4(12.5%)	0.003
Intravenous hormone n(%)	4(28.6%)	2(6.3%)	0.060
Duration of parenteral nutrition(days)	42.1±12.1	25.6±10.2	0.000
Length of hospital stay(days)	58.6±17.6	34.0±11.1	0.000
Corrected gestational age at discharge	36.2±1.2	34.6±1.0	0.000

Table 1-2 The Basic Maternal Characteristics Between BPD and Non-BPD Group

	BPD group(n=14)	Non-BPD group(n=32)	P-value
Maternal age(years)	30.7±3.5	33.5±4.1	0.065
Caesarean Section n(%)	10(71.4%)	29(90.6%)	0.173
Antenatal steroids n(%)	10(71.4%)	17(53.1%)	0.246
Premature rupture of membranes (PROM) n(%)	5(35.7%)	17(53.1%)	0.023
intrauterine infection n(%)	2(14.3%)	7(21.9%)	0.700

Table 1-3 The Comparison of FeNO and Eosinophilia Between BPD and Non-BPD Group

	BPD group (n=14)	Non-BPD group(n=32)	P-value
D1-3 Eosinophilia n(%)	3(21.4%)	0(0%)	0.050
D7-14 Eosinophilia n(%)	2(14.3%)	6(18.7%)	1.000
D21-28 Eosinophilia n(%)	7(50%)	12(37%)	0.043
FeNO(ppb)	13.9 ± 6.2	8.2 ± 3.3	0.006

Table 2 The Cytokines of the Patients

	BPD group(n=14)	Non-BPD group(n=32)	P-value
CYTOKINES pg/ml M(P25-P75)			
Day 1-3			
IFN- γ	8.7(6.8-18)	8.7(5.8-14.0)	0.582
IL-10	2.6(1.8-4.6)	3.1(1.3-3.8)	0.043
IL-12p70	1.2(0.45-2.2)	0.6(0.4-0.9)	0.454
IL-13	5.7(5.1-11.1)	7.2(4.7-10.9)	0.896
IL-1 β	1.5(0.9-14.1)	1.4(0.9-9.2)	0.940
IL-2	2.7(1.0-4.1)	1.7(1.0-3.8)	0.680
IL-4	0.33(0.2-0.7)	0.25(0.1-0.6)	0.217
IL-6	6.6(3.8-7.4)	7.4(3.8-12.9)	0.830
IL-8	176(137-370)	159(108-271)	0.895
TNF- α	0.5(0.3-1.1)	0.20(0.1-1.2)	0.370
CYTOKINES pg/ml M(P25-P75)			
Day 7-14			
IFN- γ	21.1(4.2-26.2)	11.5(9.1-17.6)	0.721
IL-10	2.4(1.6-8.1)	3.2(1.1-7.4)	0.036
IL-12p70	0.79(0.36-2.51)	0.58(0.38-1.15)	0.897
IL-13	5.8(4.7-9.3)	5.2(4.0-8.8)	0.537
IL-1 β	1.95(0.78-2.35)	1.5(0.82-2.74)	0.062
IL-2	2.28(0.76-6.54)	1.39(0.66-3.46)	0.516
IL-4	0.43(0.11-1.63)	0.27(0.14-0.92)	0.673
IL-6	7.91(2.90-22.03)	5.2(3.1-8.1)	0.033
IL-8	240.9(89.7-363.3)	126(87-339)	0.001
TNF- α	0.9(0.2-2.1)	0.4(0.2-2.9)	0.007
CYTOKINES pg/ml M(P25-P75)			
Day 21-28			
IFN- γ	9.8(5.6-17.3)	17.4(11.3-26.2)	0.150
IL-10	2.3(2.1-4.3)	2.9(2.25-4.41)	0.008
IL-12p70	0.67(0.41-1.01)	0.90(0.53-1.14)	0.426
IL-13	7.2(4.5-10.4)	9.1(4.6-10.7)	0.488
IL-1 β	2.86(1.69-3.77)	2.15(0.58-3.31)	0.042
IL-2	1.87(0.87-2.09)	1.94(0.74-2.98)	0.739
IL-4	0.44(0.12-1.18)	0.20(0.085-1.18)	0.002
IL-6	9.52(2.34-5.81)	5.45(2.27-13.0)	0.036
IL-8	459(116-301)	255.7(69.3-499.6)	0.005
TNF- α	1.2(0.98-1.78)	0.6(0.2-1.5)	0.002

Table 3 Multivariate Logistic Regression of BPD Influencing Factors

	OR	95%CI	P-value
gestational age<30 weeks	7.472	3.021-17.325	0.000
birth weight<1000g	11.265	3.134-24.325	0.000
PDA	5.388	2.216-11.103	0.000
Duration of Mechanical Ventilation (days) M	1.893	1.342-2.768	0.001
Duration of Invasive ventilation(days)	2.243	1.583-2.893	0.001
D21-28 Elevated Eosnophils	3.562	0.621-11.437	0.001
FeNO	2.547	1.397-4.824	0.000
D7-14 IL-6	1.133	1.008-1.451	0.026
D7-14 IL-8	1.092	1.021-1.438	0.031
D21-28 IL-4	1.245	1.127-2.824	0.000
D21-28 IL-6	1.276	1.091-1.671	0.001
D21-28 IL-8	1.325	1.146-1.872	0.020
D21-28 TNF- α	1.219	1.105-1.584	0.025

Figures

Figure 1

The IL-1 β between BPD and Non-BPD group on D1-3, D7-14 and D21-28(#p > 0.05; *p < 0.05)

Figure 2

The IL-4 between BPD and Non-BPD group on D1-3, D7-14 and D21-28(#p > 0.05; *p < 0.05)

Figure 3

The IL-6 between BPD and Non-BPD group on D1-3,D7-14 and D21-28(#p > 0.05; *p < 0.05)

Figure 4

The IL-8 between BPD and Non-BPD group on D1-3,D7-14 and D21-28(#p > 0.05; *p < 0.05)

Figure 5

The TNF-α between BPD and Non-BPD group on D1-3,D7-14 and D21-28(#p > 0.05; *p < 0.05)

Figure 6

The IL-10 between BPD and Non-BPD group on D1-3, D7-14 and D21-28(*p < 0.05)