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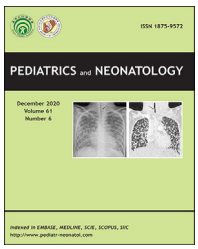

Original Article

Decreased neutrophil levels in bronchopulmonary dysplasia infants

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Key Words
bronchopulmonary dysplasia;
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preterm infant

Background: Previous studies have indicated that inflammation plays an important role in the occurrence and development of bronchopulmonary dysplasia (BPD); however, there were rare researches about the changes of neutrophils and their influence on the prognosis of BPD. Hence, we aimed to explore the changes in the number of peripheral blood neutrophils (PBNs), and the relationship between these changes and susceptibility to pulmonary infection among children with BPD.
Methods: Firstly, the gene expression of lung tissues and the number of PBNs were respectively detected by RNA sequencing and complete blood count in the 85% O_2-induced BPD model rats. Then it was analyzed the number of PBNs after birth and the incidence of pneumonia within 6 months of corrected age (CA) after discharge among full-term infants (FTIs: gestational age [GA] between $37^{0/7}$ and $41^{6/7}$ weeks, n = 88), preterm infants with (PTIs-BPD: GA <32 weeks, n = 35) or without BPD (PTIs-nBPD: GA <32 weeks, n = 41).
Results: The levels of S100A8 and S100A9 mRNAs were significantly decreased in the lungs of BPD rats. Moreover, the number of PBNs was also decreased in BPD rats. The number of PBNs at birth in FTIs was significantly greater than that in PTIs-BPD or in PTIs-nBPD ($p < 0.001$), while those between PTIs-BPD and PTIs-nBPD showed no significant difference ($p > 0.05$). Although the peripheral blood neutrophils decreased overall after birth in both PTIs-nBPD and PTIs-BPD groups, only the reduction in the PTIs-BPD group was significant ($p < 0.001$). Importantly, at 36–37 weeks of postmenstrual age (PMA), the number of PBNs in PTIs-BPD was significantly fewer than that in PTIs-nBPD ($p < 0.001$). In addition, PTIs-BPD had a significantly higher incidence of

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pneumonia than PTIs-nBPD within 6 months of CA after discharge ($p < 0.01$).
Conclusion: The number of PBNs in PTIs-BPD decreased progressively when compared to that in PTIs-nBPD, which might contribute to their susceptibility to pulmonary infection.

Abbreviations:

BPD	bronchopulmonary dysplasia
BW	birth weight
CA	corrected age
CBC	complete blood count
FTIs	full-term infants
GA	gestational age
MLI	mean linear intercept
MVD	microvessel density
NIH	the National Institutes of Health
PBNs	peripheral blood neutrophils
PMA	postmenstrual age
PND	postnatal day
PTIs	preterm infants
PTIs-BPD	preterm infants with BPD
PTIs-nBPD	preterm infants without BPD
RAC	radial alveolar count

1. Introduction

Bronchopulmonary dysplasia (BPD) was first reported in 1967 by Northway.[1] Although neonatal care technology has developed rapidly since then and the survival rate of premature infants has greatly increased, the incidence of BPD remains high.[2,3] Children with BPD are more likely to have respiratory problems than those without BPD,[3] such as readmission due to lower respiratory tract infection,[3–5] chronic pulmonary insufficiency,[3] and even increased risks of asthma and chronic obstructive pulmonary disease in adulthood.[6,7] Therefore, BPD can significantly increase family health costs and severely influence quality of life.[8] Currently, there is no effective prevention or treatment methods, thus, managing BPD, especially severe BPD, remains very difficult.[9]

Although the pathogenesis of BPD remains unclear, it is generally believed to be caused by genetic susceptibility to various pathogenic factors, such as hyperoxia, mechanical ventilation, infection, etc. These pathogenic factors can cause compound lung injury that combines physical, biological and chemical lung injuries. Inflammation plays an important role during the occurrence and development of BPD. Intrauterine infections, such as chorioamnionitis, are an important cause of BPD.[10] Furthermore, oxygen and mechanical ventilation used during BPD treatment can induce and aggravate inflammatory injuries.[11] Many inflammatory factors, including TNF-α, IL-1, IL-6, and IL-8, can be detected in the tracheal aspirate of very premature infants with BPD.[12–14] Several large clinical studies have confirmed that the early use of steroids in extremely premature infants can reduce the incidence of BPD.[15,16] Nevertheless, the causes, types, characteristics and outcomes of inflammation in BPD need to be investigated further.

Herein, we established a hyperoxia BPD model to look for the differentially expressed genes that may be involved in the development of BPD. Unexpectedly, we found significant changes of S100A8 and S100A9, two abundantly expressed genes in neutrophils. Based on these findings, we further explored neutrophil changes in BPD and their influence on the prognosis of BPD.

2. Materials and methods

2.1. Animal experiments

2.1.1. Experimental protocol

The establishment of rat BPD model followed the procedure used by Braun et al.[17] Twenty neonatal rats born naturally (within a 1 h birth time difference) were randomly assigned to a 21% O_2 group (control group, n = 10) and an 85% O_2 group (BPD group, n = 10) within 6 h after birth. An oxygen generator (Shenzhen Deda Kangjian Co., Ltd., Shenzhen, Guangdong, China) was used to continuously supply oxygen to the chamber. In addition, an oxygen-control instrument (Zhejiang Jiande Meicheng Electrochemical Analysis Instrument Factory, Jiande, Zhejiang, China) was utilized to continuously monitor the oxygen concentration, which was maintained at 85% ± 1%. The animal experimental procedures followed the Guidelines for the Care and Use of Laboratory Animals.

2.1.2. Specimen collection and processing

On postnatal day 14 (PND14), the neonatal rats were euthanized. Venous blood was rapidly collected from the right ventricle for the complete blood count (CBC). The right lung was quickly separated and placed in an Eppendorf tube. After immediate freezing in liquid nitrogen, the lungs were stored in a −80 °C freezer for RNA sequencing and RT-PCR detection. The left lung was inflated with 4% paraformaldehyde through the left main bronchus at 20 cm of H_2O pressure until it was evenly expanded. Then, it was fixed in 10% formalin overnight and embedded in paraffin. Four-micrometer serial sections were prepared for hematoxylin-eosin (H&E) and immunohistochemistry (IHC) staining.

2.1.3. Lung development evaluation

H&E and CD34 IHC staining were performed using conventional methods (anti-CD34, Ab81289, Abcam, Cambridge, UK; diluted 1:300). The radial alveolar count (RAC) and mean linear intercept (MLI) were measured on H&E section

(100 × magnification), while microvessel density (MVD) was assessed on CD34 IHC section (200 × magnification), as described elsewhere.[18–20]

2.1.4. RNA sequencing

Freshly isolated rat lung tissue was rapidly frozen in liquid nitrogen and ground up. TRIzol reagent was added to fully lyse the tissue for total RNA extraction. RNA quality was detected using agarose gel electrophoresis. cDNA was synthesized and amplified using a SMARTer Ultra Low RNA kit (Clontech Laboratories) following the manufacturer's protocol. Amplified cDNA was processed according to the TruSeq Sample Preparation Guide, version 2 (Illumina), and it was paired end-sequenced on a HiSeq 2500 (Illumina). Fragments were aligned against the rat genome. Cufflinks software was used to calculate the number of fragments per kilobase of exons per million fragments mapped for each gene.

2.1.5. RT-PCR

Fresh frozen lung tissues stored in an −80 °C freezer were collected for RNA extraction by the TRIzol method (15,596–026, TRIzol® Reagent, Ambion, USA). RNA was reverse transcribed into cDNA according to the procedure listed in the reverse transcription reagent kit [R101-01/02, HiScript Reverse Transcriptase (RNase H), VAZYME, Nanjing, Jiangsu, China]. Real-time fluorescence quantitative PCR was performed using BIO-RAD CFX96 touch q-PCR system (CFX96, Bio-Rad, Hercules, California, USA). The final data were analyzed using the $2^{-\triangle\triangle Ct}$ method. Related primers were synthesized by Wuhan Qingke Innovation Biotechnology Co., Ltd (Wuhan, Hubei, China). The primer sequences were as follows: GAPDH: GTA TGA CTC TAC CCA CGG CA (forward), AAG ACG CCA GTA GAC TCC AC (reverse); S100A8: CTC TAC AGG GAT GAC TTC AGG A (forward), CCA CCC TTA TCA CCA ACA CAA G (reverse); S100A9: ACA TCC TGA CAC CCT GAA CAA (forward), CGT GGG TTG TTC TCA TGC AG (reverse).

2.1.6. Complete blood count

The complete blood count (CBC) was performed using an automatic hematology analyzer (BC-2800Vet, Mindray, Shenzhen, Guangdong, China).

2.2. Clinical study

2.2.1. Study design

This study retrospectively analyzed the gestational age (GA), birth weight (BW), number of PBNs at birth and at 36–37 weeks postmenstrual age (PMA) and the incidence of pneumonia within 6 months corrected age (CA) after discharge of preterm infants (PTIs) born between January 1, 2014 and December 31, 2017. In addition, the GA, BW, and number of PBNs at birth of full-term infants (FTIs) during the same period were analyzed (Fig. 1). The study protocol was conducted in accordance with the Declaration of Helsinki.

2.2.2. Inclusion criteria

1) FTIs and PTIs were admitted to our neonatal intensive care unit (NICU) and had their first CBC test within 6 h after birth.
2) FTIs, born to mothers with gestational cardiomyopathy, hypertensive disorders of pregnancy or gestational diabetes etc. had a GA between $37^{0/7}$ and $41^{6/7}$ weeks; stayed in the NICU for less than 1 week; and did not have abnormal conditions, such as organic and infectious diseases after observation.
3) PTIs had a GA <32 weeks at birth and exceeded 36 weeks of PMA when discharged from the NICU. They were divided into the preterm infants with BPD (PTIs-BPD) or without BPD (PTIs-nBPD) groups according to the National Institutes of Health (NIH) criteria.[21]
4) PTIs neither had infections nor received drugs such as glucocorticoids that might increase or decrease neutrophils during 35–37 weeks of PMA. They received a CBC test at 36–37 weeks of PMA.

2.2.3. Exclusion criteria

Patients who provided incomplete information were excluded from the study.

2.3. Statistical analysis

Pairwise comparisons of the rat experimental data were performed using t test. Each experiment was repeated at least three times. Non-parametric tests were used for clinical data analysis, while the incidence of pneumonia between children with and without BPD was compared by the chi-square test. $P < 0.05$ was considered statistically significant. GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) was used for data analysis, while Adobe Photoshop CS6 (Adobe Systems Software Ireland Ltd., Dublin, Ireland) was used for figure plotting.

3. Results

3.1. Hyperoxia resulted in growth retardation, obstruction of alveolarization and pulmonary vascular dysplasia in neonatal SD rats

On PND14, all 20 neonatal SD rats survived. The rats in the 21% O_2 group (control group) showed excellent growth and development, shiny fur, ruddy auricles, sensitive response, free movement, and regular breathing. However, the rats in the 85% O_2 group (BPD group) exhibited weight loss, dry fur, pale auricles (Fig. 2A), apathetic behavior, delayed action, and shortness of breath.

Compared with rats in the 21% O_2 group, the lungs of rats in the 85% O_2 group were pale and dull (Fig. 2B). Under light microscopy, the 21% O_2 group not only showed a greater quantity of well-formed alveoli, which were uniform in size and distribution, but also had dense pulmonary microvessels, whereas the 85% O_2 group showed simplification of lung structure with a decreased quantity of alveoli, a simplified alveolar structure, an enlarged

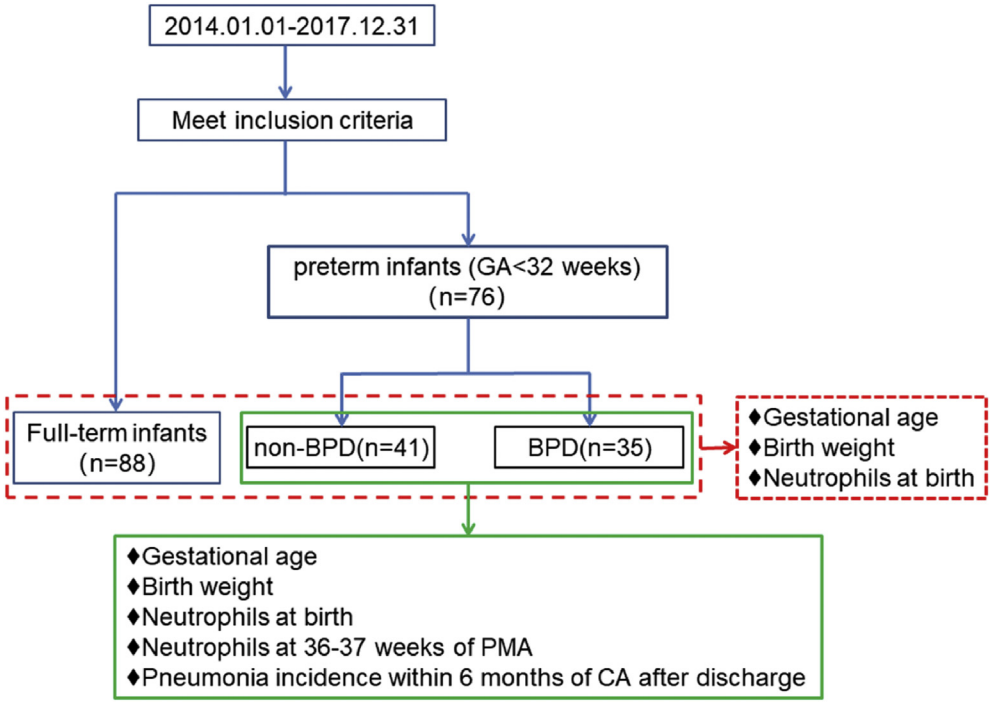

Fig. 1 Flow diagram of the clinical study. CA, corrected age; GA, gestational age; PMA, postmenstrual age.

alveolar cavity and sparse pulmonary microvessels (Fig. 2C and D).

Statistical analysis showed that the birth weight and body weight on PND7 were not significantly different between the two groups, but the body weight (Fig. 2E, $p < 0.05$), RAC (Fig. 2F, $p < 0.001$) and MVD (Fig. 2H, $p < 0.001$) on PND14 were lower in the 85% O_2 group than in the 21% O_2 group, whereas MLI showed the opposite trend (Fig. 2G, $p < 0.001$).

3.2. Hyperoxia downregulated the expression levels of lung S100A8, S100A9 mRNAs and the number of PBNs in neonatal SD rats

To study the gene expression changes in the lung tissue of the rat BPD model, we separately selected one lung specimen from the 21% O_2 and 85% O_2 groups on PND14 for RNA sequencing. Then we found that the levels of S100A8 and S100A9 mRNAs in the lung tissues from the 85% O_2 group were significantly lower than those from the 21% O_2 group (21%O_2-PND14 vs. 85%O_2-PND14: S100A8 mRNA 115.274 vs. 18.0271; S100A9 mRNA 251.448 vs. 38.1515), which was further confirmed by RT-PCR (Fig. 3A, $p < 0.001$; Fig. 3B, $p < 0.001$). Due to the abundant expression of S100A8 and S100A9 in neutrophils, above results implied that there were fewer neutrophils infiltrating the lung tissues in the 85% O_2 group than in the 21% O_2 group. Furthermore, we analyzed the number of PBNs between the two groups on PND14 and found that the number of PBNs was significantly fewer in the 85% O_2 group than in the 21% O_2 group (Fig. 3C, $p < 0.001$).

3.3. The number of PBNs gradually decreased in preterm infants with BPD

In order to investigate the number of PBNs in human newborns, we enrolled 88 FTIs, 41 PTIs-nBPD and 35 PTIs-BPD. Patient characteristics of gestational age, birth weight and gender are listed in Table 1.

Fig. 4A and B shows that the differences of gestational age and body weight between PTIs-BPD and FTIs were more significant than those between PTIs-BPD and PTIs-nBPD. This result suggested that in contrast to FTIs, PTIs-nBPD were a more suitable reference to PTIs-BPD. In Fig. 4C, the number of PBNs at birth in FTIs was significantly more than that in PTIs-BPD or in PTIs-nBPD ($p < 0.001$). However, those between PTIs-BPD and PTIs-nBPD showed no significant difference ($p > 0.05$). Although the peripheral blood neutrophils in both PTIs-nBPD and PTIs-BPD groups decreased overall after birth (Fig. 4D), only the reduction in the PTIs-BPD group was significant (Fig. 4D, $p < 0.001$). Importantly, at 36—37 weeks of PMA, the number of PBNs in the PTIs-BPD group was significantly lower than that in the PTIs-nBPD group (Fig. 4D, $p < 0.001$). These results suggested that the number of PBNs gradually decreased in PTIs-BPD after birth as compared to PTIs-nBPD.

3.4. The incidence of pneumonia within 6 months of CA after discharge was higher in PTIs-BPD than in PTIs-nBPD

Neutrophils play an important role during infection defense. We analyzed the incidence of pneumonia within 6 months of CA after discharge in the aforementioned PTIs-

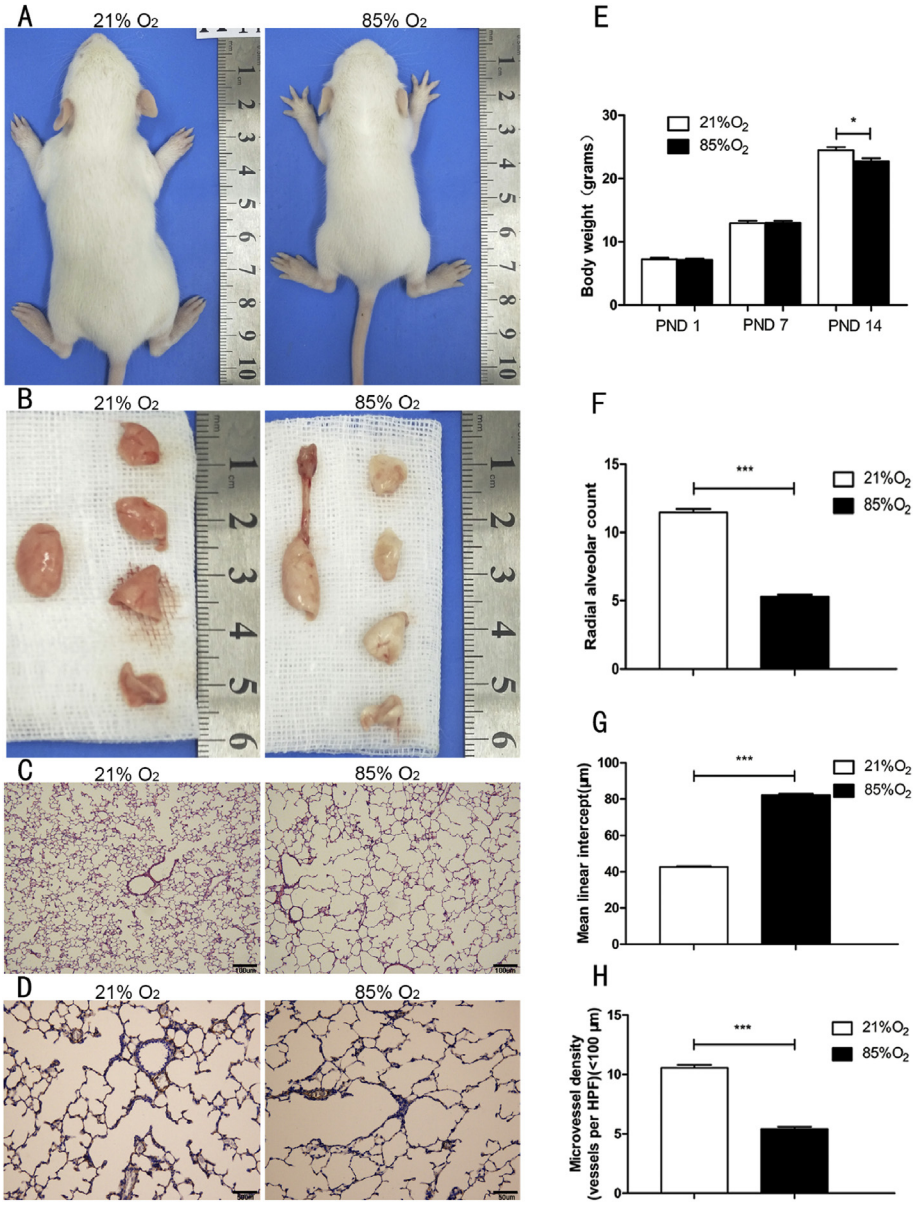

Fig. 2 The effect of hyperoxia (85% O_2) on lung development in neonatal rats. **A, B, C** and **D** show the appearance of rats (**A**), the appearance of lungs (**B**), H&E-stained lung tissue sections (**C**: original magnification × 100. Bars = 100 μm) and CD34 immunohistochemistry of lung sections (**D**: original magnification × 200. Bars = 50 μm) of 14-day-old newborn rats from the 21% O_2 and 85% O_2 groups; **E** represents the body weight comparison between the newborn rats of these two groups on PND 1, 7 and 14. **F, G** and **H** indicate the comparisons of radial alveolar count (RAC) (**F**), mean linear intercept (MLI) (**G**), and pulmonary mean microvessel density (MVD) (**H**) of the rats from these two groups on PND14. (* = $P < 0.05$, *** = $P < 0.001$) PND 1, postnatal day 1; PND 7, postnatal day 7; PND 14, postnatal day 14.

nBPD and PTIs-BPD. It was found that PTIs-BPD (11/35, 31.4%) were more easily affected by pneumonia than PTIs-nBPD (3/41, 7.3%) (Fig. 4E, $p < 0.01$).

4. Discussion

In this study, we established a BPD model using neonatal SD rats via the hyperoxia inhalation method. Compared with rats in the 21% O_2 (control) group, neonatal SD rats in the 85% O_2 (BPD) group exhibited not only body weight loss, dry fur, insufficient activity, and oxygen dependency, but also pale and dull lungs, which showed that the alveolar structure was simplified, the size of the alveoli was uneven, and some of the alveoli were fused together. Furthermore, the alveolar cavity expanded, while the number of alveoli and the density of the pulmonary micro-vessels decreased by PND14. All of these results not only reflected the clinical

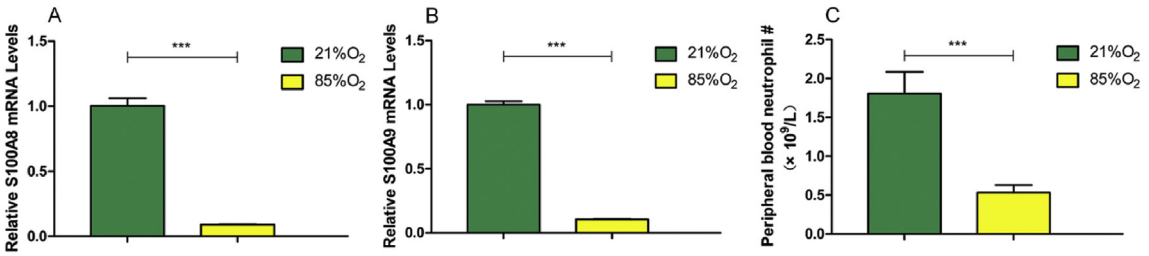

Fig. 3 The effects of hyperoxia (85% O2) on the expression of S100A8 and S100A9 mRNAs in lung tissue and the number of PBNs in neonatal SD rats. Comparisons of S100A8 mRNA (**A**) and S100A9 mRNA expression levels (**B**) in the lungs and the number of PBNs (**C**) of newborn rats on PND14 between the 21%O_2 group and the 85%O_2 group. (*** = $P < 0.001$).

Table 1 Demographics of the patients.

	FTIs (N = 88)	PTIs-nBPD (N = 41)	PTIs-BPD (N = 35)
Gestational age, week	38.57 (37.00 −41.86)	31.86 (29.00 −31.86)	30.00 (26.43 −31.86)
Birth weight, g	3,080 (1,770 −5,340)	1,520 (1,060 −2,170)	1300 (950 −2,180)
Male	47 (53.4%)	24 (58.5%)	17 (48.6%)

Values are expressed as median (range) or number (%).
FTIs, full-term infants; PTIs-nBPD, preterm infants without BPD; PTIs-BPD, preterm infants with BPD.

characteristics of the chronic respiratory insufficiency associated with BPD, but also exhibited the pathological features of alveolar differentiation and pulmonary vascular dysfunction related to BPD. Therefore, this model may simulate the characteristics of BPD and lay the foundation for further research.

Through RNA sequencing, we found that the expression of many genes in the lung tissues of SD neonatal rats exposed to 85% O_2 changed by PND14. What intrigued us was the significant reduction of S100A8 mRNA and S100A9 mRNA. As the constitutive proteins of neutrophils, both S100A8 and S100A9 were primarily derived from neutrophils and accounted for approximately 40% of their total proteins.[22] The above results implied that the number of neutrophils in the lung tissue of SD neonatal rats exposed to 85% O_2 might decrease at PND14. S100A8 and S100A9 are indicative indicators, but not specific indicators of the level of neutrophils. Moreover, by IHC staining of neutrophils in lung tissue, positive cells are not necessarily neutrophils. Therefore, we did not detect the protein levels of S100A8 and S100A9 in the lung tissue of neonatal SD rats, nor did we perform IHC staining of neutrophils in lung tissue. We further investigated the number of PBNs in children with or without BPD (PTIs-BPD vs. PTIs-nBPD). As the results showed, there was no significant difference between the two groups at birth. Although the peripheral blood neutrophils in both PTIs-nBPD and PTIs-BPD groups showed an overall decrease after birth, only PTIs-BPD had a significant downtrend. In addition, the PTIs-BPD had significantly fewer PBNs at 36−37 weeks of PMA than PTIs-nBPD. These results indicated that the number of PBNs in PTIs-BPD gradually decreased after birth when compared to that in PTIs-nBPD. Moreover, the PBNs of SD rats exposed to 85% O_2 on PND14 were also significantly lower than those in the 21% O_2 group, further confirming the clinical observation above.

There are two possible mechanisms for the decrease in peripheral blood neutrophils in children with BPD. The first one is blockage of hematopoietic stem cell differentiation. Hematopoietic stem cells in bone marrow are in a hypoxic environment, and they are generally considered to meet their energy needs through the anaerobic glycolysis pathway. Hence, they have a low oxygen demand.[23] Long-term oxygen therapy in children with BPD might disrupt the hypoxic environment of bone marrow hematopoietic stem cells, further inhibiting their proliferation and differentiation, thereby eventually affecting the number of PBNs. The other possibility is that the hyperoxic environment is adverse to the activity of neutrophils. Some evidence has indicated that the hypoxic environment stimulates neutrophil degranulation.[24] Moreover, hyperbaric oxygen reduces adhesion molecule expression on the surfaces of neutrophils, thus inhibiting their aggregation to the inflammation site.[25]

Neutrophils are critical innate immune cells and play an important role in infection defense. When the body experiences inflammation or infection, neutrophils are attracted to the inflammation site by chemokines. As phagocytosis begins, large numbers of reactive oxygen species and lysosomal enzymes are produced and released by neutrophils to decompose phagocytic tissue fragments and bacteria, killing pathogens to prevent further proliferation of pathogens and inflammation spread.[26] As the number of neutrophils decreases, the risk of infection increases significantly.[26]

The respiratory system, with a large internal alveolar surface area, directly communicates with the external environment, existing in an open state. All these factors increase its chance of exposure to the external environment compared with other human organs. Thus, it is more vulnerable to pathogen invasion. The immune system (including neutrophils) plays an important role in the defense of the respiratory system. If its function decreases (e.g., reduced peripheral blood neutrophil levels), pathogens will find it easier to invade through the respiratory tract, greatly increasing the probability of pulmonary infection. Many studies,[3–5] along with our research, have shown that children with BPD have a significantly higher incidence of pulmonary infection than those without BPD.

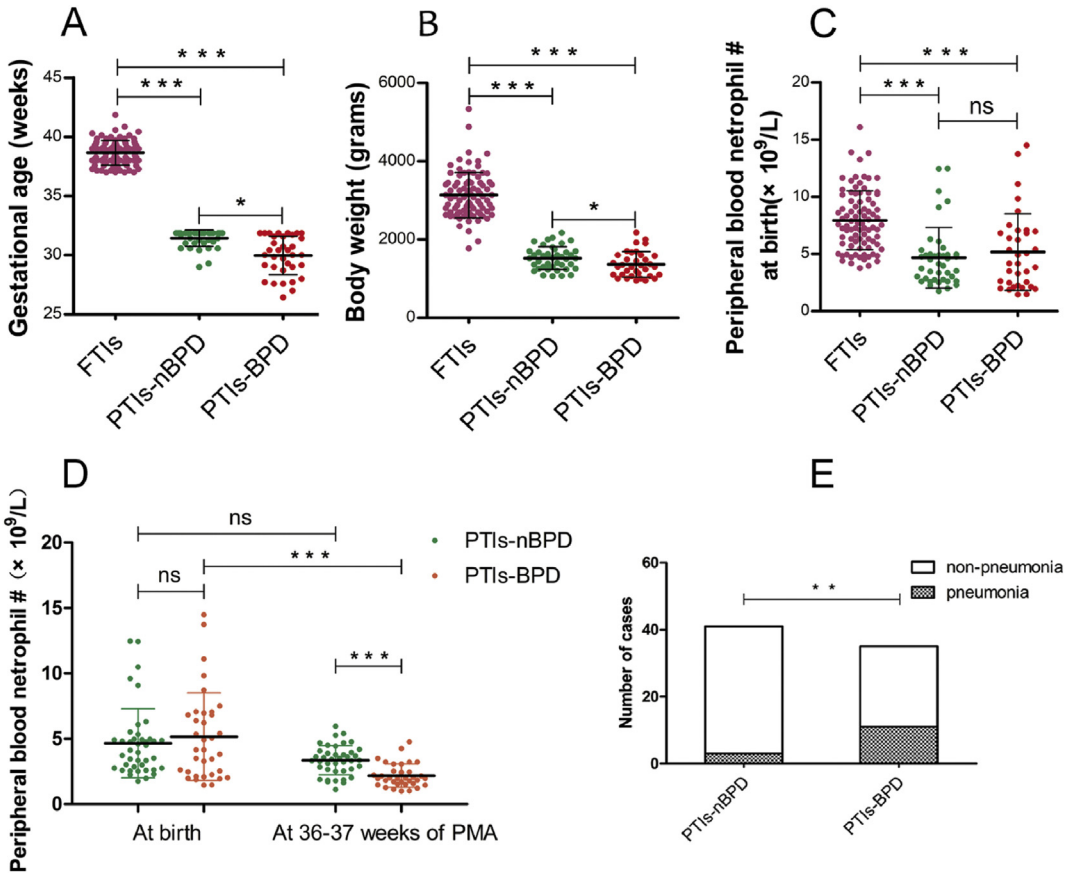

Fig. 4 The results of the clinical study. **A**, **B**, and **C** show the comparisons of gestational age (**A**), birth weight (**B**) and number of PBNs at birth (**C**) among FTIs (n = 88), PTIs without BPD (GA <32 weeks, n = 41) and PTIs with BPD (GA <32 weeks, n = 35); **D** and **E** represent the comparisons of the number of PBNs at birth and at 36—37 weeks of postmenstrual age (**D**) and pneumonia incidence within 6 months of CA after discharge (**E**) between the non-BPD group (n = 41) and the BPD group (n = 35) in PTIs (GA <32 weeks, n = 76). (ns = $P > 0.05$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). FTIs, full-term infants; PTIs, preterm infants; PTIs-BPD, preterm infants with BPD; PTIs-nBPD, preterm infants without BPD.

Repeated respiratory infections can cause multiple blows to the already fragile lung tissue of children with BPD, thereby reducing their quality of life and even becoming life threatening in severe cases.[8] According to our results, the number of PBNs in children with BPD gradually decreased with the prolongation of oxygen therapy, which could increase the likelihood of pneumonia within 6 months of CA after discharge. Thus, clinicians should pay attention to these phenomena.

In conclusion, our animal experiments and clinical studies revealed that long-term oxygen therapy reduced the number of PBNs in BPD. This might be associated with changes in the hypoxic bone marrow microenvironment caused by hyperoxia, thereby inhibiting the hematopoietic function of bone marrow. However, the reduction in PBNs might increase the incidence of pneumonia within 6 months of CA after discharge among children with BPD.

Animals

Two SPF-grade Sprague—Dawley (SD) pregnant rats were purchased from the Experimental Animal Center of Hubei University of Medicine (experimental animal license number: SCXX (E) 2016—0008, Hubei, China).

Ethical approval

The animal experimental protocol was approved by the Laboratory Animal Welfare Ethics Review Committee of Hubei University of Medicine. The Ethics Committee of Shiyan Taihe hospital approved the review of patient medical records.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

1. Northway Jr WH, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med* 1967;**276**:357—68.
2. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in care practices, morbidity, and mortality of extremely preterm neonates, 1993—2012. *JAMA* 2015;**314**:1039—51.
3. Cheong JLY, Doyle LW. An update on pulmonary and neurodevelopmental outcomes of bronchopulmonary dysplasia. *Semin Perinatol* 2018;**42**:478—84.
4. Akangire G, Manimtim W, Nyp MF, Noel-MacDonnell J, Kays AN, Truog WE, et al. Clinical outcomes among diagnostic subgroups of infants with severe bronchopulmonary dysplasia through 2 years of age. *Am J Perinatol* 2018;**35**:1376—87.
5. Acuña-Cordero R, Sossa-Briceño MP, Rodríguez-Martínez CE. Predictors of hospitalization for acute lower respiratory infections during the first two years of life in a population of preterm infants with bronchopulmonary dysplasia. *Early Hum Dev* 2018;**127**:53—7.
6. Moschino L, Stocchero M, Filippone M, Carraro S, Baraldi E. Longitudinal assessment of lung function in survivors of bronchopulmonary dysplasia from birth to adulthood. The Padova BPD study. *Am J Respir Crit Care Med* 2018;**198**:134—7.
7. Martinez FD. Early-life origins of chronic obstructive pulmonary disease. *N Engl J Med* 2016;**375**:871—8.
8. Parad RB, Davis JM, Lo J, Thomas M, Marlow N, Calvert S, et al. Prediction of respiratory outcome in extremely low gestational age infants. *Neonatology* 2015;**107**:241—8.
9. Gien J, Kinsella JP. Pathogenesis and treatment of bronchopulmonary dysplasia. *Curr Opin Pediatr* 2011;**23**:305—13.
10. Matsumura H, Ichiba H, Ohnishi S, Saito M, Shintaku H. Histologic chorioamnionitis, amniotic fluid interleukin 6, krebs von den lungen 6, and transforming growth factor β 1 for the development of neonatal bronchopulmonary dysplasia. *Jpn Clin Med* 2017;**8**:1179066017696076.
11. Shahzad T, Radajewski S, Chao CM, Bellusci S, Ehrhardt H. Pathogenesis of bronchopulmonary dysplasia: when inflammation meets organ development. *Mol Cell Pediatr* 2016;**3**:23.
12. Balany J, Bhandari V. Understanding the impact of infection, inflammation, and their persistence in the pathogenesis of bronchopulmonary dysplasia. *Front Med (Lausanne)* 2015;**2**:90.
13. Perrone S, Tataranno ML, Buonocore G. Oxidative stress and bronchopulmonary dysplasia. *J Clin Neonatol* 2012;**1**:109—14.
14. Hsiao CC, Chang JC, Tsao LY, Yang RC, Chen HN, Lee CH, et al. Correlates of elevated interleukin-6 and 8-hydroxy-2'-deoxyguanosine levels in tracheal aspirates from very low birth weight infants who develop bronchopulmonary dysplasia. *Pediatr Neonatol* 2017;**58**:63—9.
15. Baud O, Maury L, Lebail F, Ramful D, El Moussawi F, Nicaise C, et al. Effect of early low-dose hydrocortisone on survival without bronchopulmonary dysplasia in extremely preterm infants (PREMILOC): a double-blind, placebo-controlled, multicentre, randomised trial. *Lancet* 2016;**387**:1827—36.
16. Bassler D, Plavka R, Shinwell ES, Hallman M, Jarreau PH, Carnielli V, et al. Early inhaled budesonide for the prevention of bronchopulmonary dysplasia. *N Engl J Med* 2015;**373**:1497—506.
17. Braun RK, Chetty C, Balasubramaniam V, Centanni R, Haraldsdottir K, Hematti P, et al. Intraperitoneal injection of MSC-derived exosomes prevent experimental bronchopulmonary dysplasia. *Biochem Biophys Res Commun* 2018;**503**:2653—8.
18. Wang Y, Yue S, Luo Z, Cao C, Yu X, Liao Z, et al. N-methyl-D-aspartate receptor activation mediates lung fibroblast proliferation and differentiation in hyperoxia-induced chronic lung disease in newborn rats. *Respir Res* 2016;**17**:136.
19. Li H, Zhu R, Qian L, Jin W, Xie J, Kang S, et al. The 50/10 oxygen-induced retinopathy model serves as a hyperoxia and hypoxia model of bronchopulmonary dysplasia. *Am J Med Sci* 2018;**355**:581—7.
20. Maturu P, Wei-Liang Y, Androutsopoulos VP, Jiang W, Wang L, Tsatsakis AM, et al. Quercetin attenuates the hyperoxic lung injury in neonatal mice: implications for bronchopulmonary dysplasia (BPD). *Food Chem Toxicol* 2018;**114**:23—33.
21. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;**163**:1723—9.
22. Giudice V, Wu Z, Kajigaya S, Fernandez Ibanez MDP, Rios O, Cheung F, et al. Circulating S100A8 and S100A9 protein levels in plasma of patients with acquired aplastic anemia and myelodysplastic syndromes. *Cytokine* 2019;**113**:462—5.
23. Zhang CC, Sadek HA. Hypoxia and metabolic properties of hematopoietic stem cells. *Antioxid Redox Signal* 2014;**20**:1891—901.
24. Hoenderdos K, Lodge KM, Hirst RA, Chen C, Palazzo SG, Emerenciana A, et al. Hypoxia upregulates neutrophil degranulation and potential for tissue injury. *Thorax* 2016;**71**:1030—8.
25. Kendall AC, Whatmore JL, Winyard PG, Smerdon GR, Eggleton P. Hyperbaric oxygen treatment reduces neutrophil-endothelial adhesion in chronic wound conditions through S-nitrosation. *Wound Repair Regen* 2013;**21**:860—8.
26. Azcutia V, Parkos CA, Brazil JC. Role of negative regulation of immune signaling pathways in neutrophil function. *J Leukoc Biol* 2017:1—13. https://doi.org/10.1002/JLB.3MIR0917-374R.