



IL-27 Levels in Bronchoalveolar Lavage Fluid in Children with Post-infectious Bronchiolitis Obliterans

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ABSTRACT

Background: Pulmonary neutrophils may play a crucial role in the development of bronchiolitis obliterans (BO) following measles virus infection. IL-27 could potentially have a negative regulatory effect on the release of reactive oxygen species and cytotoxic granules in neutrophils.

Objective: To investigate the levels of IL-27 in the bronchoalveolar lavage fluid (BALF) of children with post-infectious bronchiolitis obliterans (PIBO) and analyze the relationship between IL-27 levels and neutrophil proportions.

Methods: A total of 24 children with PIBO were recruited for the experimental group, while 23 children with bronchial foreign bodies were included in the control group. Bronchoscopic alveolar lavage was performed in both groups. The levels of IL-27 in BALF were measured using enzyme-linked immunosorbent assay (ELISA). The proportions of neutrophils in BALF were determined by smear staining. The relationship between the levels of IL-27 in BALF and the neutrophil proportions was analyzed by the Pearson test.

Results: The levels of IL-27 in BALF were significantly lower in children with PIBO compared to children with bronchial foreign bodies ($p < 0.05$). Additionally, the proportions of neutrophils in BALF were significantly higher in children with PIBO compared to children with bronchial foreign bodies ($p < 0.05$). The levels of IL-27 were negatively correlated with the neutrophil proportions in BALF in children with PIBO ($p < 0.05$), but not in children with bronchial foreign bodies ($p > 0.05$).

Conclusion: The present study suggests that a decrease in IL-27 may be associated with an increase in neutrophils in BALF and may contribute to the pathogenesis of PIBO.

Keywords: Bronchoalveolar Lavage Fluid, Children, IL-27, Post-Infectious Bronchiolitis Obliterans

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INTRODUCTION

Obstructive bronchiolitis (OB) is a chronic airflow obstruction syndrome associated with small airway inflammation (1). The etiology of BO is associated with infection, connective tissue disease, inhalation factors, bone marrow transplantation, heart and lung transplantation, and severe infection of the lower respiratory tract is the first cause of BO in children (2-4). The diagnosis of post-infectious BO (PIBO) mainly depends on the changes in respiratory symptoms, pulmonary function, and high-resolution CT (HRCT). In recent years, the use of HRCT has significantly enhanced the diagnosis rate of PIBO in children, but its etiology and pathogenesis have not been fully elucidated.

The pathogenesis of BO is related to inflammation and abnormal immune response. Adaptive immunity is the focus of BO research, but in recent years, more and more studies have shown that autoimmunity and innate immunity are also important mechanisms of BO. Increased neutrophils in bronchoalveolar lavage fluid (BALF) are important predictors of the occurrence and severity of BO after transplantation (5, 6), suggesting that innate immune cell neutrophils play a crucial role in the progression of BO. Studies in children with BO after measles infection have also shown a significant increase in neutrophils in BALF (7). Neutrophils generate defensins, reactive nitrogen species, matrix metalloproteinases, and reactive oxygen species, which cause collagen deposition, matrix degradation, fibroblast proliferation, and finally peribronchial fibrosis (8-11).

IL-27 belongs to the IL-6/IL-12 cytokine family which is composed of EB13 and IL-27p28. IL-27 displays both anti and pro-inflammatory activities in the development of multiple diseases (12, 13), but its role in the pathogenesis of BO has not been reported. IL-27 binds to its receptors (WSX-1, gp130) and acts on various cells, including B cells, T cells, and macrophages. According to

recent research, IL-27 may have a negative regulatory function in the neutrophils' production of cytotoxic granules and reactive oxygen species (14). Studies on the mouse peritonitis model induced by yeast polysaccharides have demonstrated that early IL-27 therapy can inhibit the mobilization of neutrophils in the bone marrow and their subsequent migration from the bone marrow to the site of inflammation (15). Experiments in animal models of intracerebral hemorrhage showed that IL-27 could inhibit the release of neutrophil toxic granules, but enhance the release of beneficial molecule lactoferrin (16). Supplementation of exogenous IL-27 or lactoferrin could alleviate edema and help remove intracerebral hematoma (16). At present, whether the airway inflammatory damage of PIBO is partially caused by inadequate regulation of neutrophils due to the decrease of IL-27 remains unknown. In this study, we measured IL-27 levels and neutrophil proportions in BALF and analyzed the correlation between the IL-27 levels and the neutrophil proportions in BALF in children with PIBO.

MATERIALS AND METHODS

Subjects

24 children with PIBO and 23 children with bronchial foreign bodies who were treated in the pediatric department of Hubei Maternal and Child Health Hospital from May 2019 to May 2023 were recruited for the study. All cases of the PIBO group were consistent with the PIBO diagnostic criteria (1). The exclusion criteria of the experimental group were as follows: complicated with other respiratory diseases, such as pneumonia, asthma, or lung tuberculosis; treated with antibiotics or systemic glucocorticoids in the past two weeks. All cases of the control group were diagnosed by the history of foreign body inhalation and pulmonary CT. To prevent airway inflammation caused by foreign body residue, children with foreign body inhalation

for more than one day were excluded. Hubei Maternal and Child Health Hospital's Medical Ethics Committee approved the study, and the parents or guardians of the participants provided informed consent.

Bronchoalveolar Lavage

Using an electronic bronchoscope (BF-XP290, Olympus, Japan), bronchoscope examination and alveolar lavage were performed under local anesthesia of respiratory mucosa. Children with PIBO were given 10 ml 37°C sterilized saline to lavage the right middle lobe or the left lingual lobe according to the location of the lesion indicated by pulmonary CT. Children with bronchial foreign bodies were given 10 ml 37°C sterilized saline to lavage the opposite side of the foreign bodies at the right middle lobe or the left lingual lobe after the foreign bodies were removed. The recovery rate of alveolar lavage fluid was more than 40%.

Detection of IL-27 in BALF

After collecting the BALF, it was centrifuged for five min at 4°C and 4000 rpm. To use an enzyme-linked immunosorbent assay (ELISA) for measurement, the supernatant was kept at -80°C. IL-27 levels in BALF were detected by ELISA under the manufacturer's protocols (IL-27 ELISA kits, Elabscience, Wuhan, China). The minimal detectable concentration was 18.75 pg/ml for IL-27. All samples were measured in duplicate.

Detection of the Proportion of Neutrophils in BALF

The recovered BALF was centrifuged for 5 min at 4000 rpm and 4°C. 50 µl sediment was preserved and mixed well, and then 5~10 µl of it was used for smear. Slides were stained with May-Gruenwald Giemsa (Jiancheng, Nanjing, China). The proportion of neutrophils, eosinophils, macrophages, and lymphocytes was recorded for analysis.

Statistical Analysis

SPSS software version 25.0 (SPSS Inc.,

Chicago, IL, USA) was conducted for statistical analysis. Means±standard deviation (SD) was displayed for normally distributed continuous variables. The independent sample t-test was performed for comparison between the two groups. The Chi-square test was used to compare the sex distribution between the two groups. Correlations were analyzed by the Pearson test. $p < 0.05$ was considered as significant.

RESULTS

Clinical Characteristics of the Subjects

The current study included 24 PIBO children, 19 of whom were boys and 5 were girls. The children with PIBO ranged in age from 6 months to 8 years, with an average age of 42 months. Within the PIBO group, 9 individuals have previously been infected with adenovirus, 3 Mycoplasma pneumonia, 3 measles virus, 2 respiratory syncytial virus, and 1 rhinovirus. 6 children were not found to be infected with specific pathogens. Patchy ground-glass opacity and mosaic perfusion were suggested on pulmonary CT in most children. 23 children with bronchial foreign bodies for the control group were recruited. No significant differences in sex, age, and infection indicators were found between the two groups, as shown in Table 1. Typical pulmonary CT images of the two groups are shown in Fig. 1.

Levels of IL-27

The IL-27 levels in BALF in children with PIBO were significantly lower than those in children with bronchial foreign bodies ($p=0.000$, Fig. 2A). Further grouping according to the disease severity in the PIBO group (transcutaneous oxygen saturation <90% is considered severe and transcutaneous oxygen saturation $\geq 90\%$ is considered mild or moderate), no significant difference in the levels of IL-27 between the two subgroups was found ($p=0.491$, Fig. 2B).

Table 1. Clinical and demographic characteristics of the subjects

Characteristics	PIBO (n=24)	Control (n=23)	p-value
Sex (male/female)	19/5	14/9	0.170
Age (months)	42.88±23.50	35.96±17.64	0.261
WBC (×10 ⁹)	6.67±1.48	7.07±1.64	0.378
NEU (×10 ⁹)	3.90±0.61	4.22±1.07	0.286
LYM (×10 ⁹)	2.17±0.57	2.24±0.59	0.715
CRP (mg/l)	2.09±0.98	1.96±0.80	0.619
PCT (ng/ml)	0.20±0.10	0.22±0.11	0.496

Data are presented as means±standard deviation. WBC: white blood cell counts; NEU: neutrophils; LYM: lymphocytes; CRP: C-reactive protein; PCT: procalcitonin

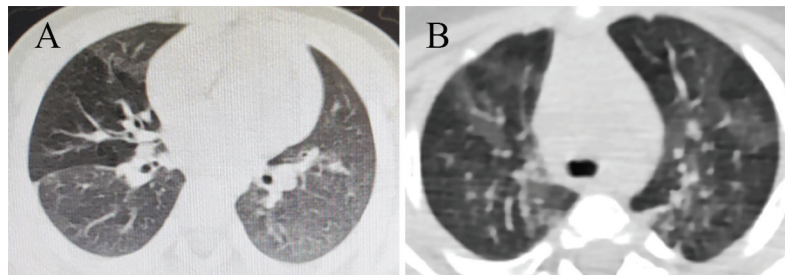


Fig. 1. Pulmonary CT images of bronchial foreign bodies (A, gas retention due to foreign bodies in the middle lobe of the right lung) and post-infectious obstructive bronchiolitis (B, bilateral mosaic perfusion).

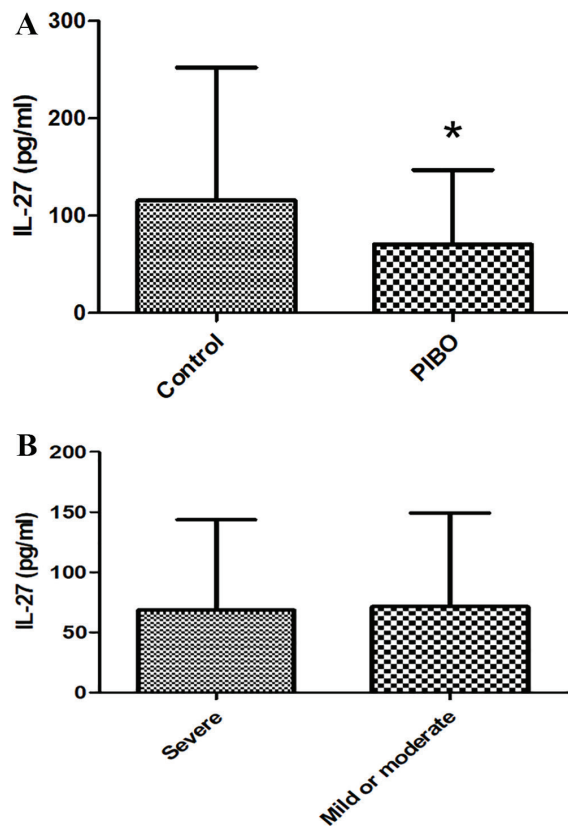


Fig. 2. (A) Comparison of the concentrations of IL-27 in BALF in children with bronchial foreign bodies (the control group) and children with PIBO (experimental group). * $p < 0.05$ versus the control group. (B) Comparison of the concentrations of IL-27 in BALF in children with severe PIBO and in children with the mild or moderate PIBO.

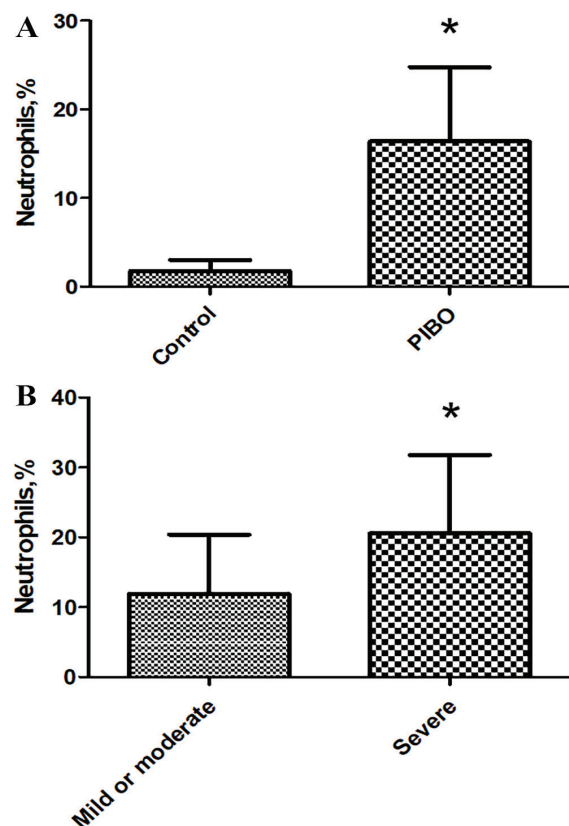


Fig. 3. (A) Comparison of the proportions of neutrophils in BALF in children with bronchial foreign bodies and in those with PIBO. * $p < 0.05$ versus the control group. (B) Comparison of the proportions of neutrophils in BALF in children with the mild or moderate PIBO and in those with severe PIBO. * $p < 0.05$ versus the mild or moderate PIBO.

Proportions of Neutrophils

The proportions of neutrophils in BALF in children with PIBO were significantly higher than those in children with bronchial foreign bodies ($p=0.000$, Fig. 3A). As shown in Fig. 3B, the proportions of neutrophils in BALF in children with severe PIBO were higher than those with mild or moderate PIBO ($p=0.011$).

Correlations of the Levels of IL-27 and the Proportions of Neutrophils

The IL-27 levels correlated negatively with the neutrophil proportions in BALF in children with PIBO ($r=-0.547$, $p=0.006$, Fig. 4A), but not in children with bronchial foreign bodies ($r=-0.102$, $p=0.643$, Fig. 4B).

DISCUSSION

In the present study, we demonstrated that the IL-27 levels decreased and the neutrophil proportions increased in BALF in children with PIBO. There were negative correlations between the levels of IL-27 and the proportions of neutrophils in BALF. Our results suggest that decreased levels of IL-27 may be associated with increased neutrophils in BALF and participate in the pathogenesis of PIBO.

The symptoms of PIBO include wheezing, prolonged cough, and hypoxia, which have a detrimental impact on health and life quality (17). The PIBO development has been reported to be related to the measles virus, adenovirus, influenza virus, Mycoplasma pneumonia (MP), respiratory syncytial virus (RSV), human immunodeficiency virus, pertussis virus, herpes simplex virus, varicella virus, etc. (2-4). Consistent with these reports, in this study, it was found that most children with PIBO had infections with adenovirus, MP, measles virus, and RSV. The pathogenesis of PIBO has not been elucidated at present. Koh et al. confirmed a significant increase of neutrophils and IL-8 in BALF in children with PIBO (7), which suggested that neutrophils may be effector cells in the pathogenesis of PIBO.

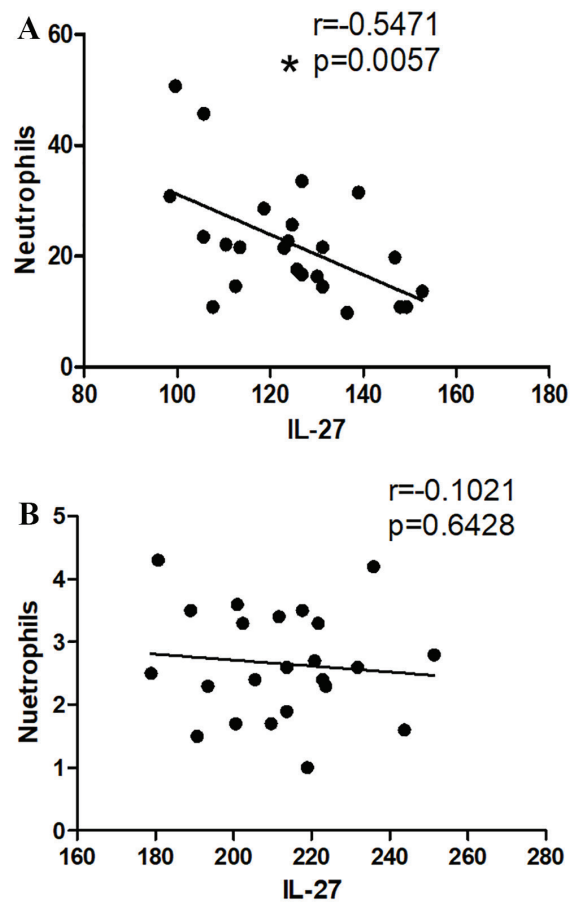


Fig. 4. Correlations of the levels of IL-27 and the proportions of neutrophils in children with PIBO (A) and in those with bronchial foreign bodies (B). Data were analyzed by the Pearson test. r =Pearson correlation. $*p<0.05$.

It was also found that neutrophils elevated in the BALF of patients with BO after lung transplantation (6). In addition, there was a correlation between the neutrophil elevation in BALF and the stage of BO secondary to lung transplantation (5). Consistent with these findings, the present study suggested that the neutrophil proportions in BALF in children with PIBO were significantly higher than those in children with bronchial foreign bodies (the control group).

IL-27 not only can promote inflammatory response by initiating Th1 cell differentiation but also reduce inflammatory response by inhibiting Th2 and Th17 cell differentiation (18). IL-27 may play a negative regulatory role in the release of reactive oxygen species and cytotoxic granules in neutrophils (14, 19). At present, the role of IL-27 in PIBO has not been reported.

Our study found that the IL-27 levels in BALF in children with PIBO were significantly lower than those in children with bronchial foreign bodies (the control group), and the IL-27 levels negatively correlated with the neutrophil proportions in BALF in patients with PIBO. Our results suggested that decreased IL-27 levels may participate in the airway inflammation of PIBO possibly due to its inadequate regulation of neutrophils. Research reveals that the quantity of neutrophils recruited to the peritoneal cavity upon zymosan application and the frequency of neutrophils in the blood significantly decreased after early administration of recombinant IL-27 (15). It is necessary to explore the therapeutic effect of IL-27 on BO in animal experiments. Whether exogenous IL-27 can alleviate airway inflammation of PIBO remains to be further explored.

Our research has encountered certain limitations. The current study's sample size is relatively small, and a larger clinical study with a more extensive sample size is necessary to validate the identified role of IL-27 in the pathogenesis of PIBO. In addition, research demonstrates that IL-27 increases on the first day of infection (20), while it cannot be completely ruled out that the higher IL-27 in the control group may be partially affected by infection factors, although children with foreign body inhalation time more than 1 day were excluded and there were no differences in infection indicators between the two groups.

CONCLUSIONS

We found decreased levels of IL-27 in BALF in children with PIBO and a close correlation between the expression of IL-27 and neutrophils. Our results suggested that IL-27 may play a crucial role in the pathogenesis of PIBO.

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Not applicable.

AUTHORS' CONTRIBUTION

WL and YZ performed all the experiments and analyzed the data. WL and YZ contributed equally to this work. XC designed the study and wrote the manuscript. All authors read and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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