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Association of IL-8 levels in serum and bronchoalveolar lavage fluid with sputum emboli in children with lobar pneumonia

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ABSTRACT

To provide a clinical reference for the management of lobar pneumonia (LP) by analyzing the association of interleukin-8 (IL-8) with the disease. A retrospective analysis was performed on 69 LP children (observation group, OG) and 60 healthy control children (control group, CG) who visited our hospital from January 2022 to November 2022. Fasting venous blood was drawn from the controls at admission to determine IL-8 levels. In addition, IL-8 concentrations in fasting venous blood and bronchoalveolar lavage fluid (BALF) were determined in LP patients in the observation group (OG) at admission and after treatment for comparative analysis with the control group (CG). The association of serum and BALF IL-8 levels in LP children, as well as the diagnostic value of IL-8 in LP, sputum emboli, and poor prognosis, were discussed.OG showed higher serum IL-8 levels than CG. Serum IL-8 had a diagnostic sensitivity of 80% and a specificity of 70% in diagnosing LP (P<0.05). Pearson correlation coefficients showed a positive correlation between IL-8 in serum and IL-8 in BALF in OG (P<0.05). In OG, IL-8 levels increased with LP progression and decreased after treatment (P<0.05). Similarly, IL-8 was increased in children with sputum emboli, and IL-8 in BALF was more effective in diagnosing sputum emboli formation (P<0.05). Finally, IL-8 also exhibited an excellent evaluation of poor prognosis in LP children after treatment (P<0.05).IL-8 is highly expressed in serum and BALF of LP children, which has excellent diagnostic effects on the occurrence of LP and the formation of sputum emboli.

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Introduction

Lobar pneumonia (LP), or alveolar pneumonia, is one of the pneumonias caused by pathogen infection, with Streptococcus pneumoniae and Haemophilus influenzae being the most important causative bacteria (1). LP is common in children. According to the World Health Organization (WHO) statistics, the incidence of LP among people under 12 years old has reached 0.4-1.6% in 2020, showing an increasing trend year by year (2, 3). With rapid onset and severe illness, LP is clinically presented with sudden high fever, chills, chest pain, cough, rusty sputum, and dyspnea, as well as related symptoms of the digestive and nervous systems in some cases, which if not effectively controlled, can induce meningitis and other serious complications (4, 5). Currently, LP is mostly treated by macrolide antibiotics (such as azithromycin), which can effectively control disease progression. However, with its widespread application in recent years, drug resistance in children has gradually become a key issue affecting the therapeutic effect of LP (6). According to a survey, there are still about 3-5% of children with LP who still have permanent pulmonary or neurological sequelae after treatment, seriously affecting their future healthy growth (7). Therefore, it is the focus of modern clinical research to thoroughly understand the pathogenesis of LP and find a more effective prevention and treatment scheme.

Inflammatory reaction is known to be the most basic pathological process in LP initiation and progression (8). Interleukin-8 (IL-8), as a chemokine family member secreted by macrophages and epithelial cells, has been confirmed to be involved in almost all reproductive processes in mammals, which underlies the close attention paid to its role in reproductive system diseases (9, 10). As research deepens, IL-8 has been gradually found to be able to attract and activate neutrophils and stimulate local inflammatory reactions such as in the lungs and stomach (11). At present, the IL family including IL-8 has been repeatedly demonstrated to participate in the onset and progression of pneumonia (12, 13), but the research on the association of IL-8 with LP is still rare.

In order to gain a better understanding of the pathogenesis of LP, this study analyzes the relationship between IL-8 and LP, to provide credible reference and guidance for the future clinical search for new diagnosis and treatment of LP.

Materials and Methods

Research time

This study was conducted in the Department of Respiratory Medicine of our hospital from January 2022 to June 2023.

Study participants

A retrospective analysis was performed on 69 LP children (observation group, OG) and 60 healthy control children (control group, CG) who visited our hospital from January 2022 to November 2022. The study was conducted in strict accordance with the Declaration of Helsinki and

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Table 1. Baseline information of children in both groups.

Group	Age	Duration of disease (d)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	PaO_2 (%)	$PaCO_2$ (%)	Boys: Girls
CG (n=60)	6.43±2.35	5.13±1.76	125.88±14.75	74.22±12.05	77.02±6.74	52.48±2.89	38: 22
RG (n=69)	6.94±1.39	4.88±2.70	126.29±16.06	75.80±10.88	77.88±5.29	53.01±3.53	42: 27
t and χ^2	1.520	0.611	0.149	0.783	0.818	0.927	0.083
P	0.131	0.542	0.882	0.435	0.415	0.356	0.774

with the informed consent of all immediate families of the study participants. The clinical data of the two groups are shown in Table 1, and the inter-group comparison showed no evident difference in age, sex and other baseline data (P>0.05, Tab. 1).

Eligibility and exclusion criteria

OG: All the included children met the diagnostic criteria for LP (14), with an age of < 12 years, a course of disease of < 1 week, and no previous antibiotic treatment. Those with other respiratory diseases (e.g., pulmonary tuberculosis and bronchial asthma), autoimmune disorders or abnormalities, or who have received glucocorticoid, immunoglobulin, interferon and other treatments were excluded. CG: Healthy control children aged under 12, with normal physical examination results and no previous major medical history, were included.

Treatment plans

All LP children were examined by CT to confirm the presence of sputum emboli and then treated according to the doctor's advice. The children were given intravenous azithromycin (Yabao Pharmaceutical Group Co., Ltd., H20010554), 10mg/kg/d, once a day for 7 days. Subsequently, azithromycin dispersible tablets (CSPC Pharmaceutical Group Co., Ltd., H20066358) were administered orally, 10mg/kd/d, once a day for 3 days. The above treatment was one course of treatment, and all the children were treated for 2 courses with an interval of 4 days.

Detection scheme

The fasting venous blood of control children at admission and the fasting venous blood and BALF of LP children at admission and after treatment were obtained. Blood and BALF samples were then centrifuged to separate serum and supernatant, respectively, for enzyme-linked immunosorbent assay (ELISA) measurement of IL-8 following the kit (Wuhan CUSABIO Co., Ltd.) instructions.

Follow-up for prognosis

All LP children were followed up at least once a month for 6 months in the form of regular review, and the occurrence of recurrence or re-progression (both defined as adverse prognosis) during the 6-month prognosis was recorded.

Outcome measures

The difference in serum IL-8 between OG and CG was observed, and the correlation between serum and BALF IL-8 in children with LP, as well as the diagnostic value of IL-8 in LP, sputum emboli and poor prognosis, were discussed.

Statistical analyses

SPSS24.0 was employed for statistical analysis. Quantitative data, represented by ($\bar{x}$±s), were compared between groups and within groups using the independent sample t-test and paired t-test, respectively. Count data were described as [n(%)], and chi-square tests were used for inter-group comparisons. The diagnostic value was identified by the receiver operating characteristic (ROC) curve, and correlation was determined by Pearson correlation coefficients. Results were considered statistically significant when P<0.05.

Results

IL-8 was higher in OG than in CG

After testing, the pre-treatment serum IL-8 level was found to be (17.29±8.12)pg/mL in OG, higher compared with CG (P<0.05, Fig. 1A). ROC analysis showed that when the serum IL-8 was greater than 15.10 pg/mL, its sensitivity and specificity in diagnosing LP in children were 65.22% and 91.67%, respectively (P<0.05, Fig. 1B). While the IL-8 level in BALF in OG was (45.07±12.24) pg/mL, which was positively associated with serum IL-8 shown by Pearson correlation coefficient analysis ((r=0.791, P<0.05, Fig. 1C).

IL-8 increased with LP progression

In OG, serum IL-8 was the lowest in the congestion stage (n=22) of LP, followed by red hepatization stage (n=34), and was the highest in the grey hepatization stage (n=13) (P<0.05, Fig. 2A). Similarly, BALF IL-8 levels in

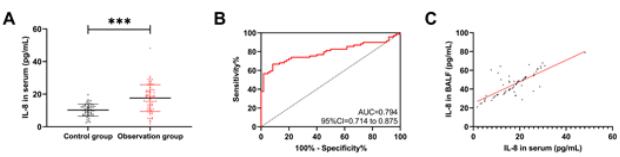

Figure 1. Comparison of IL-8 of OG and CG.A: Comparison of IL-8 in OG and CG serum. B: ROC curve for the diagnosis of LP occurrence by IL-8 in serum. C: Correlation of IL-8 in OG serum and BALF. Note: ***P<0.001.

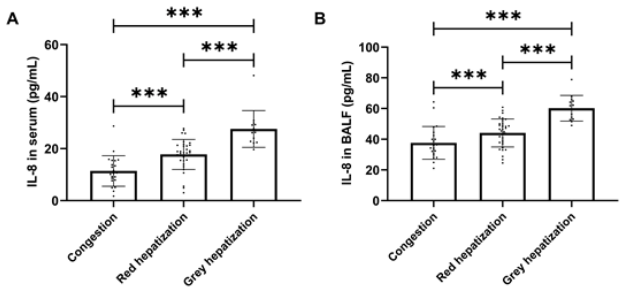

Figure 2. Comparison of IL-8 in patients with different stages of LP. A: IL-8 in LP serum at different stages. B: IL-8 in LP BALF at different stages. Note: ***P<0.001.

various LP stages from high to low were grey hepatization stage, red hepatization stage, and congestion stage successively (P<0.05, Fig. 2B).

IL-8 was higher in children with sputum emboli than in those without

Sputum emboli was present in 24 of the 69 LP children. The comparison revealed higher serum IL-8 in children with sputum emboli (21.04±9.05) pg/mL than in those without (P<0.05, Fig. 3A). According to ROC analysis, the sensitivity and specificity of serum IL-8 for the diagnosis of sputum emboli were 54.55% and 74.47%, respectively, when the serum IL-8 was higher than 20.29 pg/mL (P<0.05, Fig. 3B). Similarly, BALF IL-8 levels were elevated in children with sputum emboli compared with those without (P<0.05, Fig. 3C). The diagnostic sensitivity and specificity of BALF IL-8 for sputum emboli were 68.18% and 72.34%, respectively (cut-off > 48.39 pg/mL, P<0.05, Fig. 3D).

IL-8 decreased after treatment in LP children

After treatment, serum IL-8 in OG decreased to (14.15±4.75) pg/mL (P<0.05, Fig. 4A), while BALF IL-8 declined to (27.20±6.31) pg/mL (P<0.05, Fig. 4B).

IL-8 was higher in children with poor prognosis

During the prognostic follow-up, 11 children had adverse prognoses (6 cases developed recurrence and 5 cases experienced LP re-progression). By comparison, it can be seen that the post-treatment serum IL-8 of children with poor prognosis was (19.20±3.74) pg/mL, which was higher than that of children with normal prognosis (P<0.05, Fig. 5A). ROC analysis showed that the diagnostic sensitivity and specificity of serum IL-8 for poor prognosis were 81.82% and 81.03%, respectively, when serum IL-8 was above 16.09 pg/mL after treatment (P<0.05, Fig. 5B). Similarly, children with poor prognosis showed higher BALF IL-8 than those with normal prognosis after treatment (P<0.05, Fig. 5C). BALF IL-8 had a sensitivity of 81.82% and a specificity of 75.86% for poor prognosis (Cut-off>30.27 pg/mL, P<0.05, Fig. 5D).

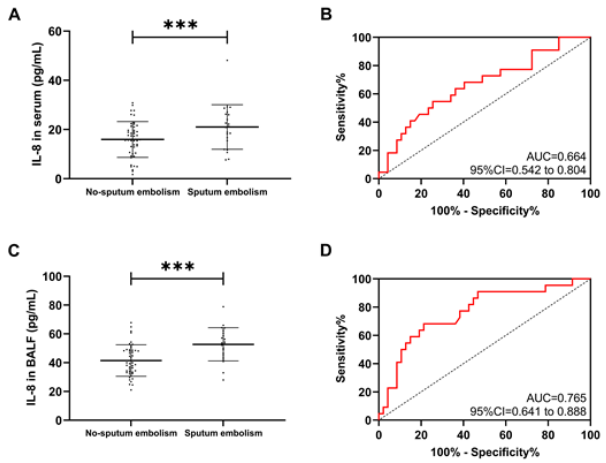

Figure 3. The relationship between IL-8 and sputum emboli. A: Comparison of IL-8 in the serum of children with and without sputum embolism. B: ROC curve for the diagnosis of sputum emboli formation by IL-8 in serum. C: Comparison of IL-8 in the BALF of children with and without sputum embolism. D: ROC curve for the diagnosis of sputum emboli formation by IL-8 in BALF. Note: $^{***}P$<0.001.

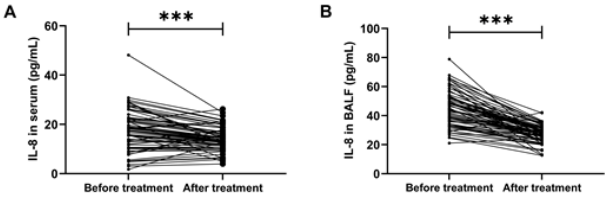

Figure 4. Changes in IL-8 before and after treatment. A: Changes in IL-8 in serum before and after treatment. B: Changes in IL-8 in BALF before and after treatment. Note: $^{***}P$<0.001.

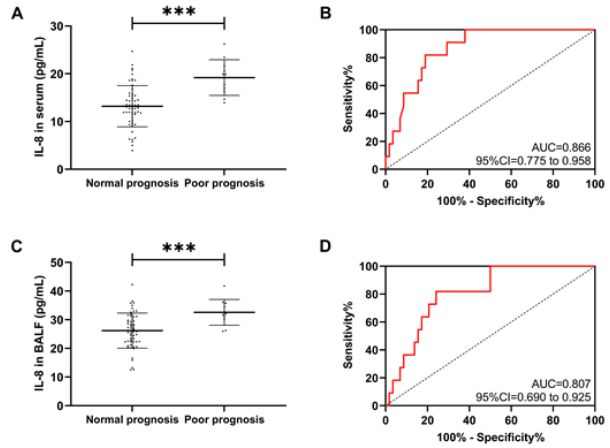

Figure 5. The relationship between IL-8 and prognosis. A: Comparison of IL-8 in the serum of children with poor and normal prognosis. B: ROC curves for the diagnosis of poor prognosis with IL-8 in serum. C: Comparison of IL-8 in the BALF of children with poor and normal prognosis. D: ROC curves for the diagnosis of poor prognosis with IL-8 in BALF. Note: $^{***}P$<0.001.

Discussion

LP, as the most common respiratory disease, is mainly caused by the decline of respiratory defense function due to decreased immunity, overwork, cold, chills, drunkenness, etc., which leads to the bacterial invasion into alveoli and the exudation of serous fluid and cellulose through the capillaries, resulting in a large number of bacteria multiplying and spreading to the entire lung through the respiratory bronchi (15). Although multiple links between the IL family and a variety of pneumonias, including LP, have been confirmed (16, 17), the relationship between IL-8 and LP is still relatively rare. In this study, we found that IL-8 also had obvious abnormal expression in LP, which undoubtedly lays a reliable foundation for finding a new diagnosis and treatment scheme for LP in the future.

First, we identified the high IL-8 expression in the serum of LP children, consistent with previous studies (18), confirming that IL-8 may be involved in the occurrence and development of LP. Second, serum IL-8 exhibited excellent diagnostic effects on LP, which also suggests its potential to be a diagnostic marker for LP. It is well known that IL-8 is mainly produced by monocytes and macrophages. Other cells, such as fibroblasts, epithelial cells, endotheliocytes and hepatocytes, can also produce IL-8 under suitable stimulation conditions (19). The molecular weight of IL-8 is about 8KD, and the main active form is 72 amino acids (20). The amino acid sequence of IL-8 has a high homology with many inflammatory factors and belongs to the same family (21). Therefore, the abnormally high expression of IL-8 can be seen not only in LP but

also in other inflammatory diseases, which also suggests the need to improve its diagnostic specificity for LP. In the BALF of children, we can see that IL-8 showed the same trend as above.

On the other hand, in children with LP of varying degrees, IL-8 levels were found to increase as the disease worsened and decreased after treatment, confirming the above results and the connection between IL-8 and LP progression. We speculate that this may be due to the rise in IL-8 levels, which promotes mitochondrial energy utilization disorders and exacerbates the abnormal transmission of oxidative respiratory chains; it may also be that the increase in IL-8 levels affects oxidative metabolic stress damage and promotes the destruction of alveolar cell integrity (22). In previous studies, D'Rozario R et al. also mentioned that IL-8 can enhance the inflammatory process by regulating the negative feedback of the body's immune function (23). Considering that LP is an inflammatory disease with immune dysfunction caused by pathogen infection, the mechanism of IL-8 may also be related to the changes in immune metabolism. However, this conjecture can not be confirmed as the immune function of children (T lymphocyte subsets, immunoglobulin, etc.) has not been tested in this study.

In addition, the formation of sputum emboli is the key to causing pulmonary ventilation dysfunction in LP, which ultimately endangers life safety (24). Although sputum emboli formation can be accurately judged by imaging means, there is no method to complete early evaluation; and instead of early preventive treatment, clinical intervention treatment can only be carried out after the formation of sputum emboli (25). Therefore, we further analyzed the relationship between IL-8 and sputum emboli formation. The results also showed elevated IL-8 in children with sputum emboli, with excellent diagnostic effects on sputum emboli formation, further suggesting that IL-8 has the potential to be an evaluation index for sputum emboli in LP children. However, IL-8 in BALF was confirmed to be more effective in the diagnosis of sputum emboli, indicating higher detection accuracy of BALF samples than blood samples. However, considering that the collection and preservation of BALF are extremely limited and not as convenient as blood samples, the detection and analysis of IL-8 in blood is still of great clinical significance.

Sputum emboli are caused by the increase of airway secretions and the damage of cilia of airway epithelial cells (26). IL-8 not only promotes neutrophil chemotaxis and enhances airway secretion viscosity, but also accelerates cellular DNA damage through chemokines, causing cell structure changes and functional disorders (27). We hypothesize that this is also the reason for the increase of IL-8 in children with sputum emboli.

Finally, we found that IL-8 was also closely related to the poor prognosis of LP and had excellent diagnostic and evaluation effects. It can be seen that in the future, we can quickly evaluate the prognosis and rehabilitation of LP children by monitoring the level of IL-8 after treatment, so as to intervene as soon as possible and provide a more reliable safety guarantee for their prognoses.

Of course, although we have a preliminary understanding of the relationship between IL-8 and LP, the regulatory role of IL-8 in LP cannot be determined by clinical trials alone. In the future, we still need to conduct more experiments to analyze the mechanism of action of IL-8, so as to develop a new LP therapy targeting IL-8. Besides, the small number of cases in this study may lead to the chance of statistical results. Moreover, longer follow-up is needed to assess the long-term prognostic significance of IL-8.

IL-8 is highly expressed in serum and BALF of LP children with excellent diagnostic effects on the occurrence of LP and the formation of sputum emboli in children, which is expected to be a clinical diagnostic index of LP. At the same time, IL-8 is closely related to the progression of LP. In the future, the disease changes and poor prognosis of LP can be evaluated by monitoring the changes of IL-8 during treatment, so as to provide a more reliable guarantee for the prognosis and safety of children.

Ethical approval
Not applicable.

Conflicts of interest
The authors report no conflict of interest.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Not applicable.

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