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ORIGINAL RESEARCH

Study on the Correlation Between the Expression of NF-ƙb in the Alveolar Lavage Fluid of Children with Severe Mycoplasma Pneumoniae Pneumonia, Its Clinical Characteristics, and Cellular Immunity

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Objective: This study explored the level of nuclear factor-ƙB (NF-ƙB) in the bronchoalveolar lavage fluid (BALF) of children with severe Mycoplasma Pneumoniae pneumonia (SMPP) and the correlation between NF-ƙB, cellular immunity, and clinical characteristics.

Methods: A total of 41 hospitalized children diagnosed with SMPP were selected and included in the SMPP group, and 13 bronchial foreign bodies (FB) without infection during the same period were included in the FB group. The NF-ƙB in the BALF of participants was detected by enzyme-linked immunosorbent assay. The correlation between NF-ƙB and laboratory findings, cellular immunity, and the clinical features in children with SMPP was analyzed. The differences in chest imaging and bronchoscopy in children with SMPP were observed.

Results: The levels of NF-ƙB were significantly increased in the SMPP group compared with the FB group ($P < 0.001$). There were correlations between different NF-ƙB pairs in the SMPP group ($P < 0.01$). Nuclear factor-ƙB (NF-ƙB) correlated with IL-6, the mycoplasma load in BALF, fever peak, length of hospital stay, and sputum suppository ($P < 0.05$). The higher the intracellular NF-ƙB level in BALF, the lower the CD3+ CD4+ value in peripheral blood ($P < 0.05$). Intracellular NF-ƙB and total NF-ƙB correlated with pleural effusion, pericardial effusion, and extrapulmonary complications ($P < 0.05$).

Conclusion: NF-ƙB is involved in airway inflammation changes in children with SMPP. The higher the level of NF-ƙB in the airway, the more severe the clinical manifestations, and the longer the length of hospital stay is likely to be.

Keywords: mycoplasma pneumoniae pneumonia, bronchoalveolar lavage fluid, NF-ƙB, cellular immunity, children

Introduction

Community acquired pneumonia (CAP) is one of the leading causes of pediatric disease and child mortality worldwide,[1] with 10% to 40% of CAP caused by Mycoplasma pneumoniae pneumonia (MPP) and about 18% requiring hospitalization; approximately 12% of hospitalized children with MPP infection need to be transferred to the intensive-care unit for further treatment.[2] The incidence of severe MPP (SMPP) has been on the rise worldwide in the past five years.[3] SMPP can cause severe lung injury, accompanied by multiple organ injury outside the lung and can be combined with multiple organ dysfunction and serious long-term sequelae, affect the prognosis and quality of life of children with SMPP, increase the burden on their families, and may even be life-threatening.[4] Therefore, the diagnosis and treatment of SMPP in children pose a great challenge to pediatricians. Within a broader scope, it also draws the attention of pediatricians in terms of how to recognize MPP early, diagnose and treat the condition in time to prevent it from developing into SMPP, and avoid complications and sequelae.

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Mycoplasma Pneumoniae (MP) is detected mainly by isolation and culture, serological specificity and non-specificity experiments, nucleic acid amplification technology, and other pathogenic techniques.[5] Treatment of MPP involves the administration of antibiotics, steroids, or intravenous immunoglobulins that disrupt protein synthesis (eg, macrolides and tetracyclines) and inhibit DNA replication (such as fluoroquinolones),[6] as well as prompt bronchoscopy and bronchoalveolar lavage treatment.

At present, the pathogenesis of MPP is unclear. Virulence factors of Mycoplasma pneumoniae and pathogen-mediated pathogenesis may be related to adhesion to the surface of host cells, direct cytotoxicity to host cells, immune damage induced by an inflammatory response, and immune escape.[7] Mycoplasma pneumoniae encodes a variety of virulence factors, including membrane polysaccharides, glycolipids, cohesins, community-acquired respiratory distress syndrome toxins and toxic metabolites.[8] Serum IgE levels were significantly elevated in children with MP-associated extrapulmonary diseases.[8] MP could decrease CD4 + T cells, increase CD8 + T cells, and decrease the CD4 + t/CD8 + t ratio. The more severe the disease, the more obvious the imbalance of T cells.[9] The levels of IL-6, IL-1β, IL-8 and other cytokines were significantly increased in the acute phase of SMPP. The serum levels of IgG, IgM, and IgA in patients with SMPP were also significantly increased in the acute and convalescent stages. The same antigen shared by the membrane sugar of MPP in brain and lung tissue can induce a cross-reaction, form an immune complex, amplify the autoimmune response, and give rise to multi-system immune injury.[10] Another virulence factor for MPP is lipoprotein. Mycoplasma pneumoniae recognizes Toll-like receptors (TLRs); for example, TLR1, TLR2, and TLR6 participate in inflammation by stimulating the secretion of proinflammatory cytokines, such as tumor necrosis factors (TNFs) α, IL-1β, IL-6, and other inflammatory mediators via the nuclear factor-ƙB (NF-ƙB) pathway.[11] However, proinflammatory cytokine TNF-α, IL-6, and IL-1β can trigger the NF-ƙB signaling cascade again and induce overexpression of NF-ƙB,[12] which can easily lead to SMPP. NF-ƙB is part of the Rel family, and NF-ƙB transcription factors are key dual regulators of inflammatory response and immune homeostasis and important in the pathogenesis of inflammatory diseases.[13] At present, the study of NF-ƙB is mainly focused on animal experiments; however, a few studies on NF-ƙB in MPP are related to NF-ƙB in serum, with no current research available on NF-ƙB expression in bronchoalveolar lavage fluid (BALF). Fiberoptic bronchoscopy and BALF are safe and effective in the diagnosis and treatment of MPP. Fiberoptic bronchoscopy can directly reflect pathological changes in lung tissue (such as airway mucosal edema, mucosal plica, pallor, congestion and secretion volume, color) and biochemical changes (BALF can be used for laboratory biochemical and pathogenic detection); concurrently, it can provide BALF for the study of NF-ƙB.

The aim of this study was to observe changes in the chest imaging and bronchoscopy findings in children with SMPP and then analyze the correlation between NF-ƙB in BALF, as well as the clinical features and laboratory findings of SMPP. We hope this study can provide valuable information for clinical diagnosis and treatment and improve the quality of life of children with SMPP.

Materials and Method

Participants

Children admitted to the Department of Pediatrics from October 1 to December 31 in the First Affiliated Hospital of Xinxiang Medical University were selected for the study using the convenience sampling method. This study was approved by the Hospital Ethics Committee (no. 2021021) with the informed consent of family members.

The study's inclusion criteria were as follows: ① participants younger than 14 years old; ②patients diagnosed with SMPP or with foreign body removal via bronchoscopy; ③ informed consent was provided for full participation in the study.

The exclusion criteria were as follows: ① children who did not meet the above-noted diagnosis or treatment; ② children with congenital immunodeficiency, congenital pulmonary hypoplasia, congenital heart defect, severe malnutrition, or inherited metabolic diseases; ③ SMPP accompanied by severe chronic lung diseases (eg, asthma, tuberculosis and other chronic lung diseases); ④ incomplete clinical data.

Combining relevant diagnostic criteria and consensus,[4,14–16] a diagnosis of SMPP was described as follows: ① shortness of breath (diagnostic criteria: age <2 months, respiratory rate (RR) >60/min; 2–12 months, RR ≥50/min; 1–5

years, RR ≥40/min; >5 years, RR ≥30/min). In children younger than 5 years of age, an elevated RR is indicative of pneumonia, and an RR >70 beats per minute is often indicative of hypoxemia or tachycardia, with or without dyspnea (nasal flapping, sunken sign, moaning) and cyanosis; ② hypoxemia, pulse oxygenation ≤0.92 when inhaling air; ③ chest X-ray or computed tomography (CT) scan showed that the area of lung involved was ≥2/3 or multilobar in nature; ④ pulmonary complications, such as atelectasis, lung necrosis, pleural effusion, and lung abscess occurred; ⑤ combined with other serious biological damage (eg, central nervous system infection, myocarditis, and heart failure).

Children with SMPP who met the above criteria were enrolled in the SMPP group. Children with bronchoscopic foreign body removal without acute infectious disease were enrolled in the FB group. After inclusion and exclusion, 41 cases in the SMPP and 13 cases in the FB control groups were confirmed. In the SMPP group, the patients were divided into sub-groups, based on whether there was atelectasis, phlegm suppository, pleural effusion, pericardial effusion, or extrapulmonary complications for further comparison.

General Information

The clinical data of the subjects were collected, including sex, age, hospital number, chief complaint, diagnosis, fever peak, time of fever onset before admission, duration of fever, and length of hospital stay. On the first day of admission, peripheral venous blood samples were collected at Xinxiang Medical University in the Laboratory Department of the First Affiliated Hospital. The blood routine was as follows: platelet (PLT) count, lymphocyte (LY-RRB) count, white blood cell (WBC) count, c-reactive protein (CRP) levels, lactate dehydrogenase (LDH) levels, and IL-6 levels. On the first day of admission, a throat swab was taken for mycoplasma-DNA (MP-DNA) load. Bronchoscopy and BAL therapy were performed within one week after admission. Secretion and mucosal changes were observed; MP-DNA and NF-ƙB were detected in appropriate BALF. Peripheral venous blood was taken within two weeks after admission to test the level of cellular immunity and check for various extrapulmonary complications; chest imaging examinations were performed within two weeks after the onset of the disease (for observation of, eg, degree of lung injury, pleural effusion, pulmonary consolidation, pericardial effusion, and atelectasis).

Experimental Methods

The Bronchoscopy and Lavage Procedures

According to the Chinese pediatric flexible bronchoscopy guidelines (2018),[17] fiberoptic bronchoscopy and BAL therapy were performed in the endoscopy room after relevant pre-examination preparation. The procedure was as follows: fasting 4–6 hours before the operation, general anesthesia by intravenous administration, under continuous ECG monitoring. A fiberoptic bronchoscope was directly inserted through the trachea or nasal cavity; the trachea, bilateral main bronchi, and the openings of each pulmonary segment were observed in turn. Bronchoalveolar lavage fluid was drawn out under negative pressure to detect the pathogen, and the appropriate volume of L-cysteine was injected into the affected area according to the situation. Vital signs, such as respiration, heart rate, blood pressure, and oxygenation were closely monitored during and after the operation. If vital signs become unstable at any time during the surgery, the procedure should be suspended immediately, the bronchoscope should be withdrawn, oxygen should be given, and other appropriate measures taken to assess whether the operation should be carried out after the vital signs stabilize. If the symptoms of children cannot be alleviated, the surgery should be stopped and timely treatment provided. After the operation, the vital signs of the patients should be continuously observed and serious postoperative complications, such as laryngeal edema and laryngeal spasm, should be noted.

Bronchoalveolar Lavage Treatment

A 6 mL BAL solution was centrifuged at 2500 r/min at 4°C for 5 minutes, and the supernatant was labeled for use. The calculated actual sample concentration was labeled as "Bronchoalveolar lavage fluid NF-ƙB" (BALF NF-ƙB). The precipitated cells collected at the bottom of the centrifuge tube were dispersed by hand bomb; then, 1 mL cell lysate was added and centrifuged at 5000 r at 4°C for 15 minutes. The supernatant was put into a 1.5 mL centrifuge tube and labeled for use; the actual sample concentration obtained was labeled as "intracellular NF-ƙB". The sum of NF-ƙB in BALF and intracellular NF-ƙB in the same patient was labeled "total NF- ƙB" (label notes included name, hospital number, sampling date, and disease) and stored at –20°C in a refrigerator.

Bronchoalveolar Lavage NF-Ƙb Detection

A human NF-ƙB enzyme-linked immunosorbent assay kit (Elisa Kit, Wuhan Huamei Biological Engineering Co., Ltd.) was used for detection of NF-ƙB in specimens. The instructions of the reagent was strictly followed.

The standard sample in the Elisa kit was centrifuged at 8000 rpm for 30 seconds; 1 mL of the sample dilution was added after centrifugation, and the bottom of the cryopreservation tube was repeatedly aspirated with a gun head five times to help dissolve sample. Seven 1.5 mL centrifuge tubes were arranged as S6–S0, and each tube was diluted by 250 μL of the sample solution. After dilution, the standard sample and its complex well, as well as the sample to be tested and its complex well were set, and 100 mL of the standards or sample was added to each well. After gentle shaking, the sample was placed in a 37°C incubator and incubated for 2 hours. After 2 hours, the plate was removed, the liquid in the plate was discarded, and the plate was dried. After adding 100 ML of biotin-labeled antibody to each well, the plate was incubated for 1 hour. After 1 hour, the plate was removed from the incubator, and 200 ML of washing liquid was added to each well. The plate was soaked for 2 minutes, discarded, washed three times, and dried. Then, a 100 μL Avidin-HRP solution was added to each well and incubated for 1 hour. After 1 hour, the plate was removed from Incubator. The above steps were repeated and the plate was washed five times. Then, a 90 ML substrate solution, 37°C, was added to each well in turn, avoiding light for 15–30 minutes. Next, 50 μL of the termination solution was added to each well in sequence to stop the reaction. Within 5 minutes after stopping the reaction, the optical density (OD) value of each pore was measured by a microplate reader using a wavelength of 450 nm. The average two measurements were taken to avoid error.

Experimental Data Processing

After subtracting the average of S0 hole and its complex hole from the standard value and the average of the sample value and complex hole, the standard curve was drawn. The logarithmic coordinates were as follows: horizontal coordinates for the OD value, vertical coordinates for the concentration of standards, and drawing a logarithmic standard curve. The Curve Expert (Version 1.34) professional software was used to create the standard curve for analysis.

Statistical Analysis

The SPSS Statistics (v.26.0) statistical software was used to analyze the data, which accorded with a normal distribution, and was described by means ± standard deviation ($x \pm s$). A *t*-test and median and interquartile ranges were used, as well as a nonparametric rank-sum test. Qualitative data were expressed as percentages (%) and were compared between groups using chi-square tests. Pearson or Spearman tests were used for linear correlation analysis. In univariate analysis, variables with statistical significance and those considered to have an impact on outcomes were included in a multivariate logistic regression model to explore the independent factors affecting outcomes; $P < 0.05$ indicated that the difference was statistically significant.

Results

NF-Ƙb Participates in Inflammatory Airway Changes in SMPP

There were 41 cases in the SMPP group (25 males and 16 females) and 13 cases in the FB Group (8 males and 5 females), with $P > 0.05$ in chi-square test. Compared with the FB group, the levels of NF-ƙB in BALF, intracellular NF-ƙB, and total NF-ƙB were significantly increased in the SMPP group ($P < 0.001$), suggesting that NF-ƙB is involved in the inflammatory airway changes of SMPP. See Table 1 and Figure 1 for details.

Table 1 The Level of NF-Ƙb in SMPP Group and FB Group

	SMPP	FB	*Z* value	*P* value
BALF NF-ƙB (ng/mL)	13.68 (10.53, 19.12)	2.19 (1.39, 4.69)	−5.392	<0.001
Intracellular NF-ƙB. (ng/mL)	24.50 (19.15, 36.80)	1.58 (1.23, 2.66)	−5.392	<0.001
Total NF-ƙB (ng/mL)	39.56 (30.04, 56.90)	4.40 (3.32, 6.79)	−5.392	<0.001

Abbreviations: NF-ƙB, Nuclear factor-ƙB; SMPP Severe Mycoplasma Pneumoniae Pneumonia; FB, foreign bodies; BALF, bronchoalveolar lavage fluid.

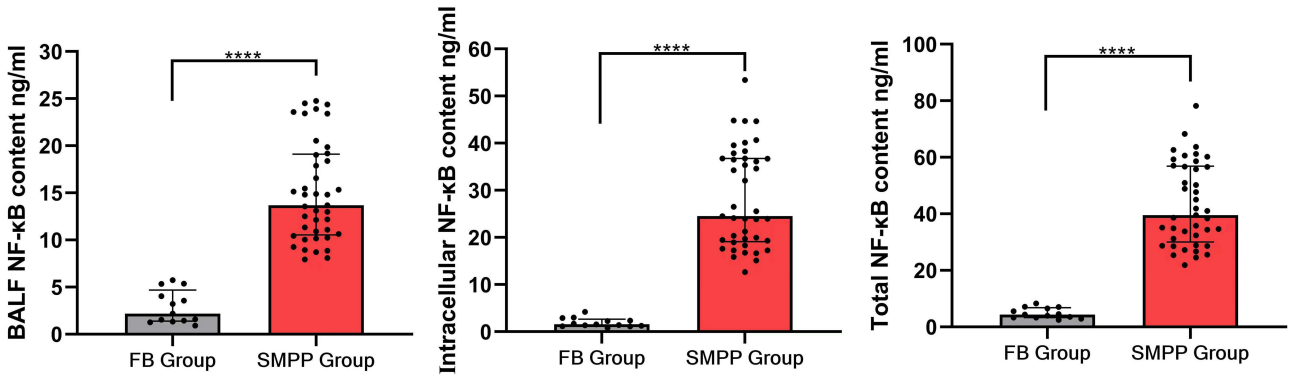

Figure 1 NF of FB group and SMPP group- ƙ Correlation of B (****$p < 0.0001$).

Analysis of the Clinical Characteristics of SMPP

The results indicated a 58.5% (25 cases) occurrence of intrapulmonary complications (14.6% [6 cases] of atelectasis, 4.9% [2 cases] of emphysema, and 51.2% [21 cases] of pleural effusion). Among them, 9.8% (4 cases) had pleural effusion and atelectasis, and 78.0% (32 cases) had a serious extrapulmonary complication (including 19.5% [8 cases] of pericardial effusion), of which 41.5% (17 cases) had both intrapulmonary and extrapulmonary complications. One patient did not have intrapulmonary or extrapulmonary complications but showed lobar pneumonia with urticaria, a repeating high fever (>39.0°C) that lasted 9 days and a significantly increased D-dimer level. No children with pleural and pericardial effusion underwent surgery (including intubation and drainage). No deaths occurred in this study. All 41 SMPP patients had a fever and cough, with a mean age of 7.1 years, a mean hospital stay of 13.15 days, and a mean fever peak of 39.4°C. See Table 2 for details. The higher the level of intracellular NF-ƙB, the lower the level of $CD3^+CD4^+$ in peripheral blood. There was no correlation between the cellular immunity of peripheral blood in the SMPP group and the levels of total NF-ƙB and NF-ƙB in BALF.

Table 2 Basic Clinical Characteristics of Children with Severe Mycoplasma Pneumoniae Pneumonia

Clinical features	n or M(P25, P75) or (X±S)
Total number of people (example)	41
Boy (example)	25
Age (years)	7.1±2.6
Days of hospitalization	13.15±5.86
Fever days before admission	8.0 (6.0, 12.0)
Total number of fever days	11.0 (7.0, 13.5)
Heat peak (°C)	39.4±0.7
BALF mycoplasma load (copies/mL)	1.73E+07 (1.57E+06, 1.63E+08)
IL-6 (pg/mL)	27.5 (11.45, 52.06)
LDH (U/L)	407.0 (267.5, 911.5)
WBC ($\times10^9$/L)	7.10 (5.45, 11.05)
LY ($\times10^9$/L)	1.55 (0.88, 2.71)
PLT ($\times10^9$/L)	289.0 (207.5, 408.5)
CRP (mg/L)	25.4 (3.57, 56.79)
BALF NF-ƙB (ng/mL)	13.68 (10.53, 19.12)
Intracellular NF-ƙB (ng/mL)	24.50 (19.15, 36.80)
Total NF-ƙB (ng/mL)	39.56 (30.04, 56.90)

Abbreviations: LDH, Lactate dehydrogenase; WBC, white blood cell; LY, lymphocyte; PLT, platelet; CRP, c-reactive protein; BALF, bronchoalveolar lavage fluid.

Table 3 Correlation Analysis Between BALF MP-DNA and Clinical Characteristics

		Age	Number of Days in Hospital	Heat Peak	Duration of Fever	IL-6	CRP	LDH
BALF MP-DNA	r P value	−0.128 0.427	0.474 0.002	0.040 0.806	0.282 0.074	0.453 0.003	0.324 0.039	0.289 0.067

Abbreviation: MP-DNA, mycoplasma-DNA;

Table 4 Analysis of Correlation Between IL-6 and Clinical Characteristics

		Age	Number of Days in Hospital	Heat Peak	Duration of Fever	MP-DNA	CRP	LDH
IL-6	*r* *P* value	0.298 0.059	0.401 0.009	0.291 0.065	0.041 0.80	0.453 0.003	0.605 0.000	0.411 0.008

Abbreviations: MP-DNA, mycoplasma-DNA; LDH, Lactate dehydrogenase; CRP, c-reactive protein.

Correlation Analysis of Clinical Features of SMPP

The higher the level of BALF in MP-DNA, the higher the level of IL-6 and CRP in peripheral blood, and the longer the hospital stay ($P < 0.05$). The higher the level of IL-6 in peripheral blood, the higher the level of CRP and LDH in peripheral blood, and the higher the level of MP-DNA in BALF, the longer the hospital stay ($P < 0.01$) was. See Table 3 and Table 4, Figure 2.

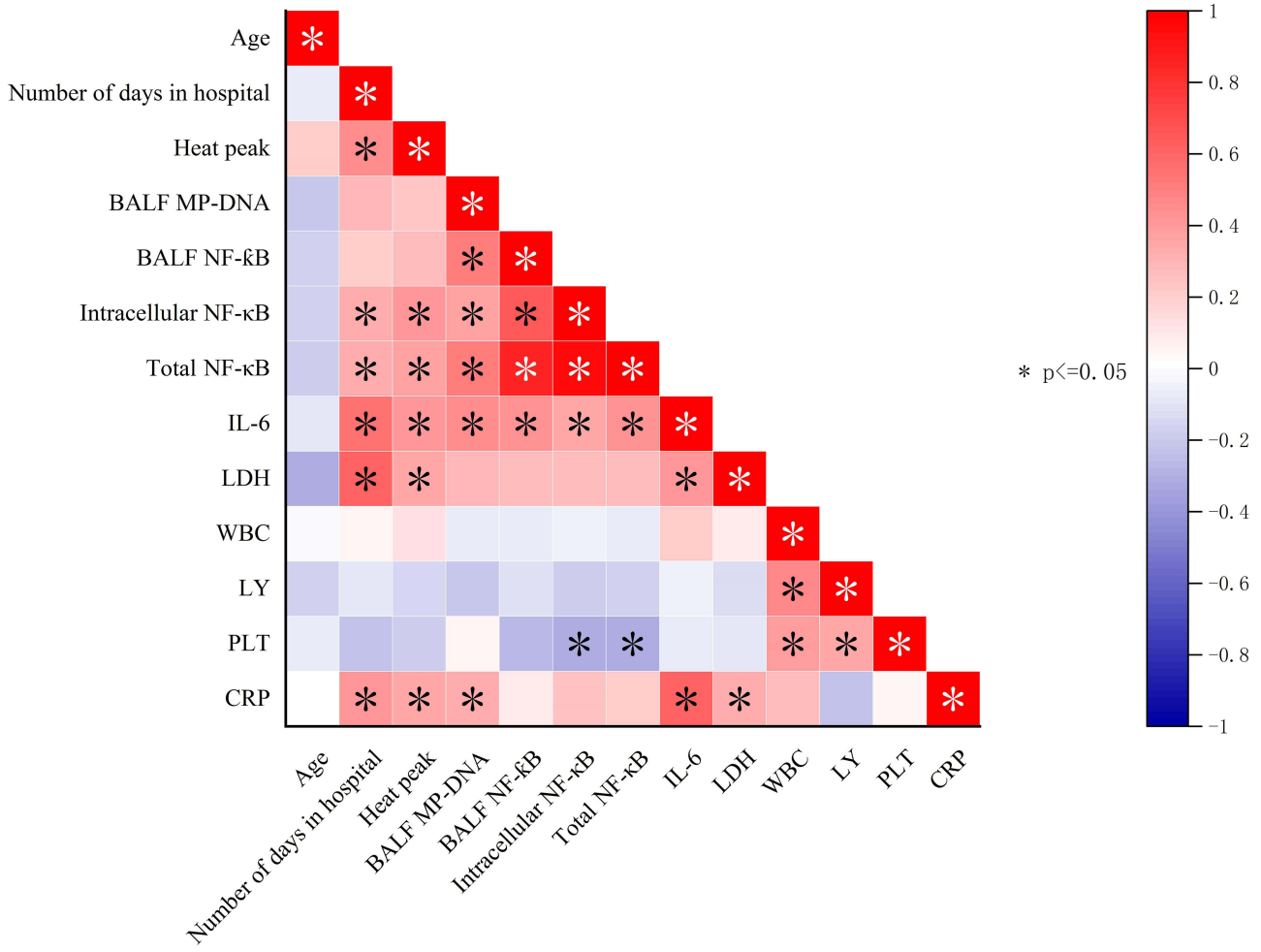

Figure 2 Correlation between clinical features (*p <0.05, red indicates positive correlation, blue indicates negative correlation).

Correlation Analysis of NF-Kb in SMPP with Clinical Features

There was a correlation between the levels of nf-κb in BALF, NF-κb in cells and total NF-κb in BALF ($P < 0.01$). The higher the level of NF-ƙB, the higher the level of BALF in MP-DNA; the higher the level of IL-6 in peripheral blood, the higher the heat peak, and the longer the hospital stay ($P < 0.05$).

The higher the level of NF-κb and total NF-ƙB in BALF, the longer the fever lasted ($P < 0.05$). There was no correlation between NF-ƙB and age, $CD3^+CD4^+/CD3^+CD8^+$, $CD3^+CD8^+$, LDH, WBC, LY, CRP, and days of fever before admission ($P > 0.05$). See Table 5 for details.

The Correlation Between NF-Kb and Sputum Suppository and Its Complications

In 41 cases of SMPP, 53.7% (22 cases) were complicated with a sputum suppository under bronchoscopy, including 9.8% (4 cases) of plastic bronchitis. The higher levels of BALF NF-ƙB, intracellular NF-ƙB and total NF-ƙB, the easier it was to form the sputum suppository ($P < 0.05$). There was no correlation between the levels of nf-κb in BALF and extrapulmonary complications, atelectasis, pleural effusion, pericardial effusion, or degree of lung injury (multilobar or unilobar lung injury; $P > 0.05$). See Table 6 and Table 7, Figure 3 for details.

Table 5 Correlation Analysis of NF-Kb in BALF, NF-Kb in Cells, Total NF-Kb and Clinical Features

	BALF NF-ƙB		**Intracellular NF-ƙB**		**Total NF-ƙB**	
	r	***P* value**	***r***	***P* value**	***r***	***P* value**
Age	−0.033	0.839	−0.145	0.364	−0.117	0.466
Heat peak	0.341	0.029	0.467	0.002	0.461	0.002
Days of hospitalization	0.365	0.019	0.445	0.004	0.453	0.003
Duration of fever.	0.398	0.010	0.297	0.060	0.375	0.016
BALF MP-DNA	0.510	0.001	0.377	0.015	0.502	0.001
BALF NF-ƙB	1		0.644	0.000	0.846	0.000
Intracellular NF-ƙB	0.644	0.000	1		0.942	0.000
Total NF-ƙB	0.846	0.000	0.942	0.000	1	
IL-6	0.433	0.005	0.347	0.026	0.430	0.005
LDH	0.271	0.086	0.261	0.100	0.280	0.077
WBC	−0.062	0.700	−0.042	0.793	−0.075	0.641
LY	−0.112	0.487	−0.189	0.235	−0.177	0.269
PLT	−0.267	0.092	−0.308	0.050	−0.312	0.047
CRP	0.087	0.591	0.256	0.107	0.220	0.168
$CD3^+CD4^+$ (n=17)	−0.145	0.580	−0.483	0.050	−0.390	0.122
$CD3^+CD8^+$ (n=17)	−0.321	0.209	0.081	0.758	−0.034	0.896
$^{CD3+CD4+}/_{CD3+CD8+}$	0.123	0.639	−0.395	0.117	−0.238	0.358

Abbreviations: LDH, Lactate dehydrogenase; WBC, white blood cell; LY, lymphocyte; PLT, platelet; CRP, c-reactive protein; BALF, bronchoalveolar lavage fluid; MP-DNA, mycoplasma-DNA.

Table 6 NF-Kb Levels in Phlegm Suppository Group and No Phlegm Suppository Group

	BALF NF-ƙB (ng/mL)	**Intracellular NF-ƙB (ng/mL)**	**Total NF-ƙB (ng/mL)**
With phlegm suppository group	16.60±5.92	31.57±11.20	48.16±15.63
No sputum suppository group.	13.24±3.83	24.64±8.54	37.89±11.29
T value	−2.115	−2.197	−2.378
P value	0.041	0.034	0.022

Abbreviations: NF-ƙB, Nuclear factor-ƙB; BALF, bronchoalveolar lavage fluid.

Table 7 Correlation Analysis Between BALF NF-Ƙb and Complications

	BALF NF-ƙB (ng/mL)	*Z value*	*P value*
Extrapulmonary Complications	14.24(10.96, 19.69)	−1.087	0.277
No Extrapulmonary Complications	13.56(8.90, 17.09)		
Atelectasis	14.63(9.91, 20.47)	−0.111	0.912
No atelectasis	13.68(10.63, 19.20)		
Pleural effusion	15.13(10.87, 21.98)	−1.122	0.262
No pleural effusion	13.27(10.19, 15.43)		
Pericardial effusion	18.55(11.55, 24.19)	−1.678	0.093
No pericardial effusion	13.58(10.21, 17.49)		
With Phlegm suppository	14.28(10.53, 21.26)	−0.704	0.482
No Phlegm suppository	12.50(10.43, 18.40)		

The higher the level of NF-ƙB in the cells, the more likely it was to be complicated with extrapulmonary complications and the more likely it was to form pleural effusion and pericardial effusion ($P < 0.05$). There was no correlation between NF-ƙB and atelectasis or lung injury ($P > 0.05$). See Table 8 and Figure 4.

The higher the level of total NF-ƙB, the more likely it was to be complicated with extrapulmonary complications and the more likely it was to form pericardial effusion and pleural effusion ($P < 0.05$). There was no correlation between total NF-ƙB and the degree of lung injury and atelectasis ($P > 0.05$). See Table 9 and Figure 5.

The Chest CT Changes of SMPP

Chest CT showed that 36.6% (15 cases) had a single lobe lung injury and 63.4% (26 cases) had multiple lobe lung injuries. See Figure 6.

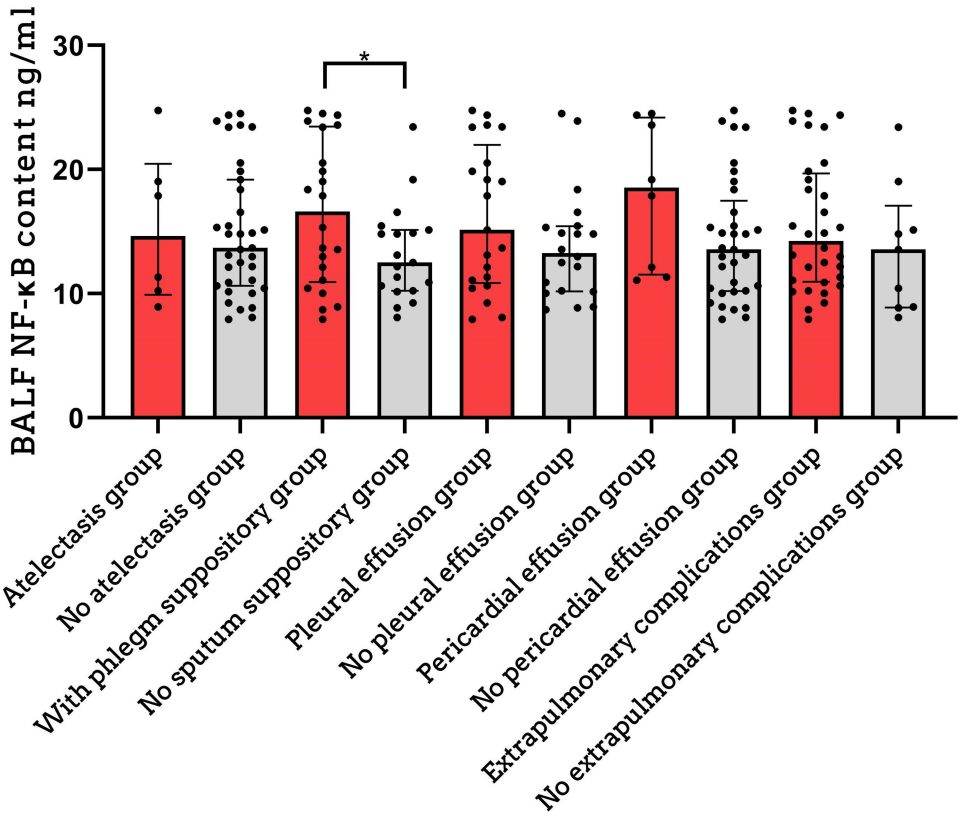

Figure 3 Correlation between BALF NF-ƙB and complications (*p< 0.05).

Table 8 Correlation Analysis Between Intracellular NF-Ƙb and Complications

	Intracellular NF-ƙB (ng/mL)	*Z value*	*P value*
Extrapulmonary Complications	33.17(21.93, 28.21)	−2.677	0.007
No Extrapulmonary Complications	19.31(17.02, 20.17)		
Atelectasis	32.58(19.81, 46.90)	−1.328	0.184
No atelectasis	24.10(18.33, 36.74)		
Pleural effusion	36.74(18.46, 40.39)	−2.282	0.022
No pleural effusion	22.57(19.10.26.30)		
Pericardial effusion	38.72(36.28, 43.52)	−3.158	0.002
No pericardial effusion	23.85(17.97, 34.99)		
With Phlegm suppository	29.30(18.89, 37.49)	−0.636	0.525
No Phlegm suppository	23.85(18.98, 36.80)		

Changes in Mucosa Under Fiberoptic Bronchoscopy

In 41 cases of SMPP, 36.6% (15 cases) showed mucosal plica, 24.4% (10 cases) showed pale mucosal edema, 75.6% (31 cases) showed hyperemia and edema, and 43.9% (18 cases) showed mucosal hemorrhage or erosion.

Mucosal hyperemia and edema were the most common (8 cases, 19.5%), followed by hyperemia + wrinkled, and hyperemia + bleeding (7 cases, 17.1%). The lowest was mucosal hyperemia and edema with pallor (1 case, 2.4%). There was no correlation between the level of NF-ƙB and mucosal changes (P > 0.05). See Table 10 and Figure 7 for details.

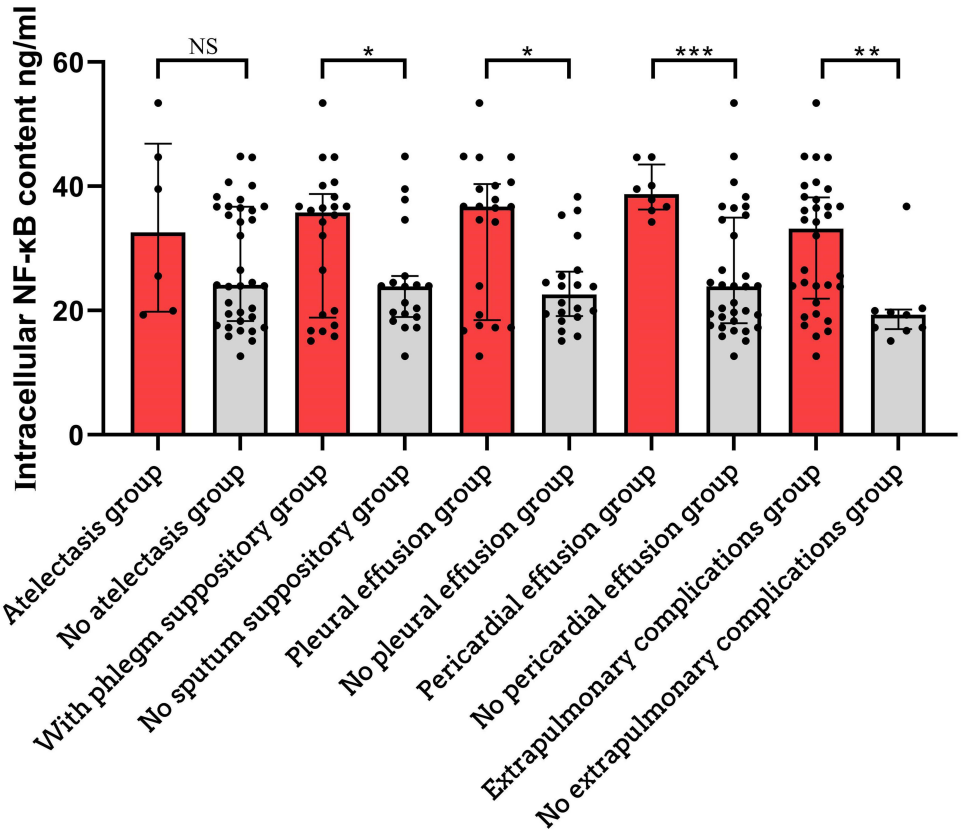

Figure 4 Correlation between intracellular NF-ƙB and complications. (NS $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Table 9 Correlation Analysis of Total NF-Ƙb and Complications

	Total NF-ƙB (ng/mL)	*Z value*	*P value*
Extrapulmonary Complications	46.38(34.42, 58.27)	−2.205	0.027
No Extrapulmonary Complications	28.91(27.89, 36.75)		
Atelectasis	44.64(34.10, 66.51)	−1.033	0.302
No atelectasis	39.56(28.69, 56.72)		
Pleural effusion	50.92(33.51, 60.71)	−2.060	0.039
No pleural effusion	35.02(28.75, 41.66)		
Pericardial effusion	57.87(52.13, 62.13)	−3.059	0.002
No pericardial effusion	35.15(28.64, 48.95)		
With Phlegm suppository	43.46(31.54, 60.32)	−1.001	0.317
No Phlegm suppository	35.15(28.59, 56.65)		

Logistic Regression Analysis of Extrapulmonary Complications in the SMPP Group

There was a significant correlation between intracellular and total NF-ƙB and extrapulmonary complications ($P < 0.05$). Only the OR and 95% Confidence interval (CI) of intracellular nf-ƙB were higher than 1, indicating intracellular nf-ƙB as being a risk factor for extrapulmonary complications. See Table 11 and Table 12 for details.

Discussion

In other studies, activated NF-ƙB was detected in the lung tissue and epithelial cells of MP-infected mice.[11,12] When Morin[12] and Baicalin[18] inhibited NF-ƙB signal transduction in experimental animals, lung inflammation was improved and lung tissue injury alleviated, indicating that NF-ƙB is involved in the inflammatory response of MPP.[11] These results were consistent with our results. Moreover, we found that, for the population where NF-ƙB is involved in the

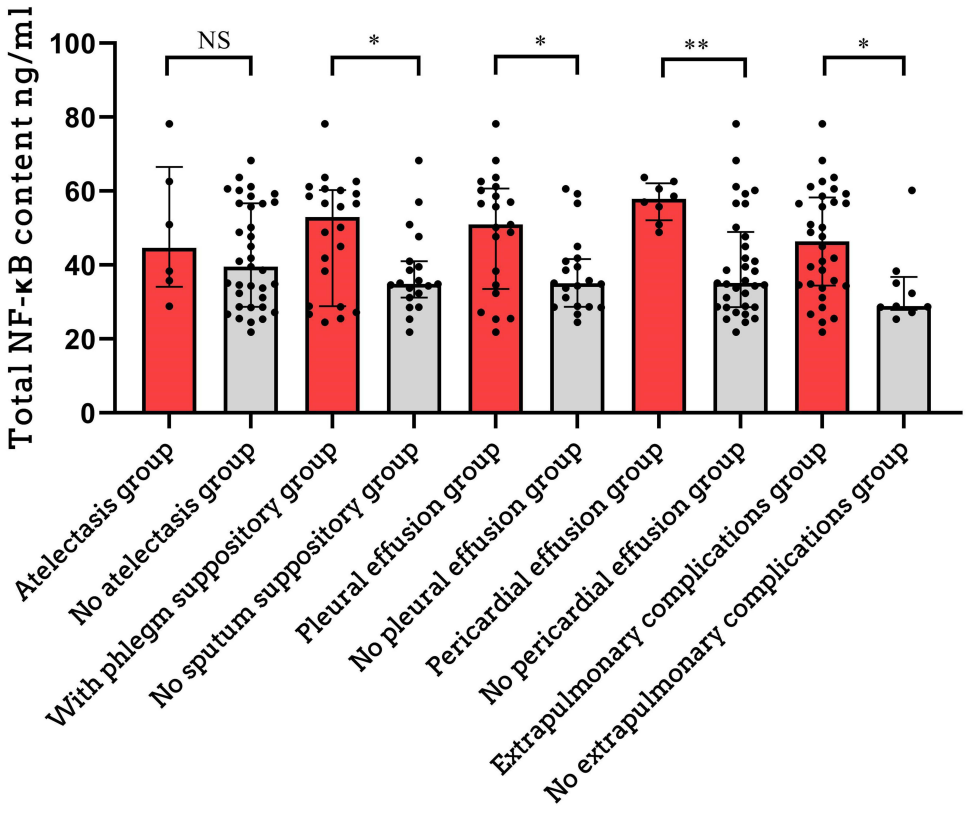

Figure 5 Correlation between total NF-ƙB and complications (NS>0.05,*p <0.05, **p <0.01).

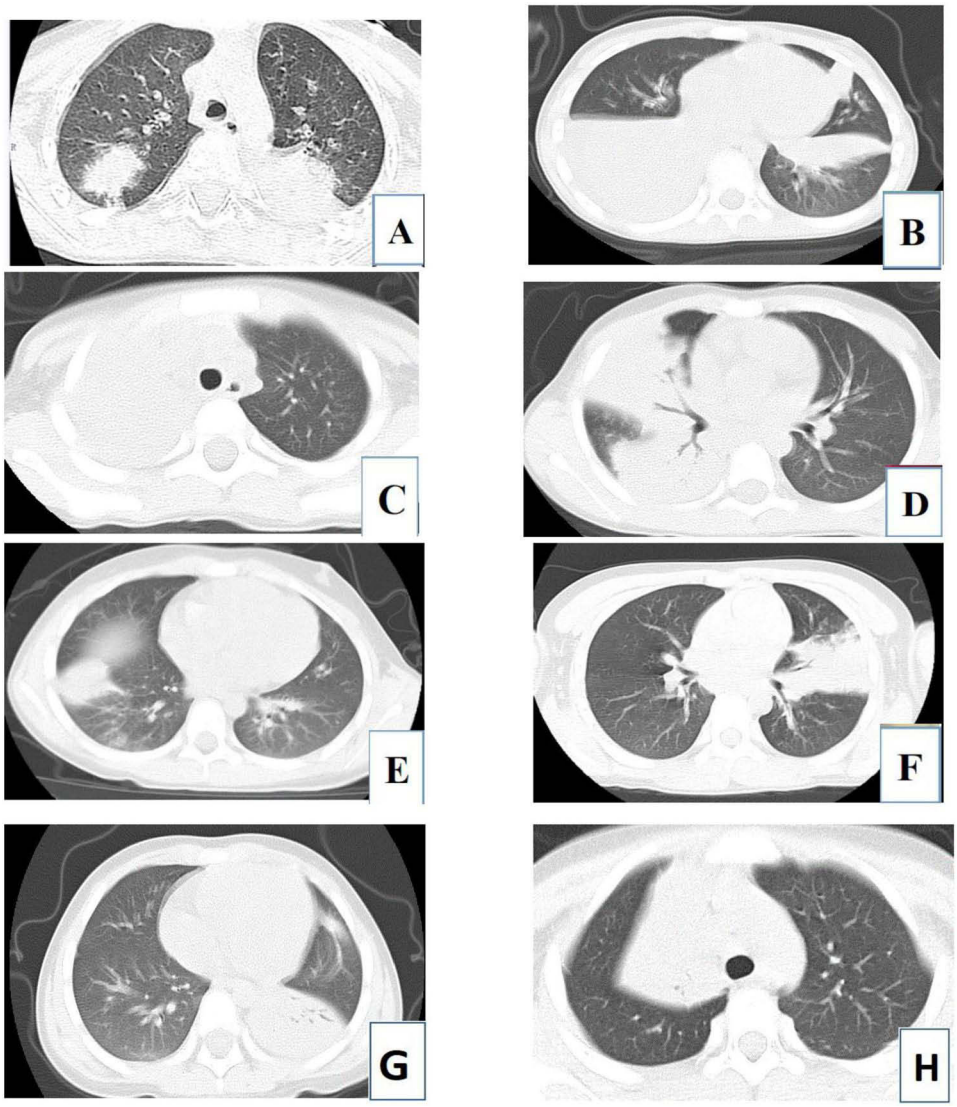

Figure 6 Chest CT changes. (**A**) Multilobar consolidation (high-density shadow, 3-year-old boy); (**B**) Consolidation of the right and left upper and lower lobes (5-year-old boy). (**C**) consolidation of the right upper lobe (high-density shadow, 4-year-old Boy); (**D**) Consolidation of the right middle lobe (hyperdense opacity, 6-year-old boy) (**E**) consolidation of the right lower lobe (high-density shadow, 9-year-old boy); (**F**) Consolidation of the left upper lobe (hyperdense opacity, 7-year-old boy). (**G**) Left lower lobe consolidation (high density, 5-year-old girl); (**H**) Atelectasis of the right upper lobe (wedge-shaped soft tissue shadow in a 9-year-old girl).

inflammatory changes in the airways of children with SMPP, and the higher the NF-ƙB in the airways, the more severe the clinical manifestations and the longer the hospital stay were.

NF-Ƙb Signal Transduction and Its Correlation with IL-6, MP and Fever

In our study, there was a positive correlation between BALF NF-ƙB, intracellular NF-ƙB and total NF-ƙB, the levels of IL-6, BALF in MP-DNA, fever peak, and length of hospital stay were significantly correlated with the levels of NF-ƙB

Table 10 Mucosa Changes Under Bronchoscopy

Mucosal Changes	Pale	Hyperemia	Pale + Wrinkled	Hyperemia + Wrinkled	Hyperemia + Pale	Pale + Bleeding	Hyperemia +Bleeding	Hyperemia + Pale + Wrinkled	Hyperemia + Pale + Bleeding	Hyperemia + Bleeding + Wrinkled
n	4	8	3	7	1	3	7	2	3	3
%	9.8	19.5	7.3	17.1	2.4	7.3	17.1	4.9	7.3	7.3

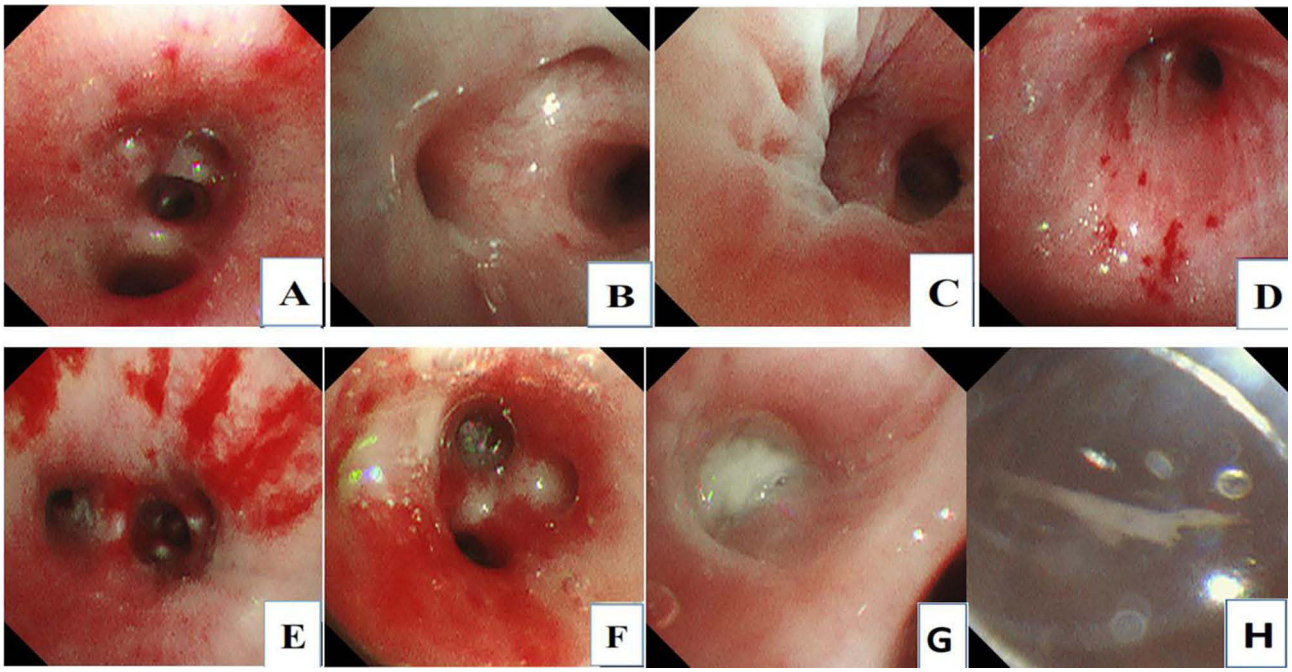

Figure 7 Mucosal changes under fiberoptic bronchoscopy. (**A**) 7-year-old girl with mucosal congestion and bleeding; (**B**) 4-year-old boy with pale mucosa; (**C**) 3-year-old girl with mucosal folds; (**D**) 11-year-old girl with mucosal bleeding points and folds; (**E**) 9-year-old boy with mucosal hemorrhage; (**F**) 5-year-old Mucous membrane erosion in a girl; (**G**) Sputum suppository in a 5-year-old boy; (**H**) Bronchial tube shape in a 4-year-old boy.

($P < 0.05$). In cases where, the higher the titer of MP in the airway, the more MP adhered to airway epithelial cells, the more MP bound to TLRs, and the higher the level of activated NF-ƙB, IL-6 could re-activate NF-ƙB and create a vicious cycle, leading to a cascade of proinflammatory cytokines, eventually leading to SMPP.[12] Additionally, IL-6 plays an important role in the pathogenesis of MPP. Zhao Yujie et al showed that IL-6 level is closely related to the severity of SMPP.[19] Compared with the FB control group, TNF-α, IL-6, IL-1β, IL-4, IFN-γ and IL-10 in BALF were significantly increased in the mild MPP group, and more significantly in the SMPP group. Dysregulated cytokines are associated with MPP severity.[20] In our study, serum IL-6 levels in the 25th percentile of the SMPP group were significantly higher than the high limit of the normal range. BALF in IL-6 was superior to blood IL-6 for predicting the severity of CAP in

Table 11 Univariate Logistic Regression Analysis of Extrapulmonary Complications

Variable	*B value*	Standard Error of b value	Wald Chi-Square value	P value	*OR value*
Age	−0.189	0.136	1.954	0.162	0.827
Sex	0.156	0.700	0.050	0.824	1.169
Heat peak	0.623	0.521	1.431	0.232	1.865
WBC	0.043	0.085	0.254	0.614	1.044
LY	0.244	0.307	0.633	0.426	1.277
PLT	−0.001	0.003	0.082	0.775	0.999
CRP	0.009	0.010	0.695	0.404	1.009
LDH	0.003	0.002	3.160	0.075	1.003
IL-6	0.018	0.015	1.541	0.215	1.019
BALF MP-DNA	0.000	0.000	0.794	0.373	1.000
BALF NF-ƙB	0.079	0.072	1.212	0.271	1.083
Intracellular NF-ƙB	0.139	0.053	6.761	0.009	1.149
Total NF-ƙB	0.078	0.033	5.485	0.019	1.081

Abbreviations: LDH, Lactate dehydrogenase; WBC, white blood cell; LY, lymphocyte; PLT, platelet; CRP, c-reactive protein; BALF, bronchoalveolar lavage fluid; MP-DNA, mycoplasma-DNA; NF-ƙB, Nuclear factor-ƙB.

Table 12 Multivariate Logistic Regression Analysis of Extrapulmonary Complications

Variable	b value	Standard Error of b value	Wald Chi-Square value	*P value*	OR value	95% CI
Age	−0.202	0.191	1.121	0.290	0.817	0.562 1.188
LDH	0.004	0.002	3.774	0.052	1.004	1.000 1.009
Intracellular NF-ƙB	0.794	0.356	4.975	0.026	2.211	1.101 4.442
Total NF-ƙB	−0.412	0.206	3.995	0.046	0.662	0.442 0.992

Abbreviations: LDH, Lactate dehydrogenase; NF-ƙB, Nuclear factor-ƙB.

hospitalized children. It has also been shown that cytokines like TNF-α, IL-1β, IL-8, and IL-6 can induce the over-expression of NF-ƙB.[12]

Correlation Between NF-Ƙb and Fever Duration

We found that the higher the level of NF-ƙB and total NF-ƙB in BALF, the longer the duration of fever. To some extent, intracellular NF-ƙB may reflect the level of NF-ƙB in vivo. For ethical reasons, no lung biopsy was performed to detect NF-ƙB in lung tissue. In theory, the higher the level of NF-ƙB, the greater the inflammation, and the longer the duration of fever; however, we found that the intracellular NF-ƙB had no relationship with fever duration. The possible causes for this were considered as follows. We studied patients with SMPP; when clinical symptoms or imaging findings were severe, the early intravenous administration of glucocorticoid inhibited the inflammatory NF-ƙB signal transduction pathway in vivo. Concurrently, the BAL treatment could eliminate a degree of MP and inflammatory factors, which may shorten the course of the fever. Kim Sh et al[21] found that children with pleural effusion had more lung consolidation, higher CRP levels, and their fever lasted longer.

The Correlation Between MPP and IL-6, CRP

We also found that IL-6 was strongly correlated with CRP ($P < 0.001$) and MP-DNA was positively correlated with CRP and IL-6 ($P < 0.05$). The higher the MP-DNA load, the more serious the infection, and the higher the CRP and IL-6 levels were. When the body is stimulated by an inflammatory response, the liver produces large amounts of CRP; this is an acute phase reactant produced in response to IL-6, which is associated with severe outcomes in pneumonia. The levels of IL-6 in BALF, CRP, and ESR in blood increased with the severity of pneumonia from mild to severe, while the levels of WBC and neutrophils in blood did not change significantly with the severity of pneumonia. CRP and BALF in IL-6 have been suggested for predicting the severity in hospitalized children with CAP.[22]

The Correlation Between Nf-Kb and Pleural Effusion, Pericardial Effusion, and Extrapulmonary Complications

We also found that intracellular nf-κb and total NF-ƙB correlated with pleural effusion, pericardial effusion, and extrapulmonary complications ($P < 0.05$); in these instances, pericardial effusion, pleural effusion and extrapulmonary complications were more likely to occur. Logistic regression analysis showed that intracellular NF-ƙB was a risk factor for extrapulmonary complications. Bourbia et al[23] found that the concentration of NF-ƙB in BALF was associated with lung inflammation, oxygen exposure, and lung outcome in intubated premature infants, and the degree of activation of nf-kb could reflect the severity of lung disease. The expression of serum NF-ƙB in the SMPP group was higher than that in mild MPP group; the expression of serum NF-ƙB in SMPP patients with pleural effusion, extrapulmonary complications, pulmonary consolidation and atelectasis were significantly higher than those without relevant manifestations.[13]

The Correlation of NF-Ƙb with Atelectasis, Lung Injury and Changes in Airway Mucosa

Nys M studies found that NF-ƙB activation may induce an increase in inflammation, and the level of NF-ƙB activation may determine the severity of respiratory dysfunction.[24] A similar trend was found in the study conducted by Kah Peng Eg et al.[25] The difference between the two groups was that the levels of NF-ƙB in BALF, intracellular NF-ƙB, and total

NF-ƙB in the atelectasis group and multi-lobar lung injury group were slightly higher than those in the non-atelectasis and single-lobar lung injury group, respectively ($P > 0.05$). There was no correlation between NF-ƙB, intracellular NF-ƙB, and total NF-ƙB in BALF and mucosal plica, mucosal edema, mucosal hyperemia and edema, or mucosal hemorrhage or erosion. Most of the mucosal changes were not a single mucosal injury, but were related to the severity of pulmonary consolidation or atelectasis in patients with severe MPP. The BALF from a severe lung injury was the main source of retained specimens. When atelectasis and consolidation were present, the BALF could not fully enter the lung injury site. Early application of glucocorticoids in severe clinical or imaging manifestations may effect the experimental results.

Correlation Between NF-Ƙb and Sputum Suppository

We found that the levels of NF-ƙB, intracellular NF-ƙB, and total NF-ƙB in BALF in the group with a sputum suppository were significantly higher than those in the group without it ($P < 0.05$), suggesting that the over-activated NF-ƙB may aggravate injury to the airway epithelia and the formation of a sputum suppository will be more likely. It has been shown that serum levels of CRP, IL-2, IL-4, IL-6, and TNF-α are significantly correlated with airway mucus thrombosis.[26] The expression of IL-6, IL-8, IL-1, TNF-α, and other downstream proinflammatory cytokines were enhanced by the activation of NF-ƙB. The bronchial group had a stronger immune response. In accordance with these findings, we found that the higher the level of NF-ƙB in BALF, the lower the $CD3^+CD4^+$ level in peripheral blood ($P < 0.05$), indicating the inferior ability of immune cells to phagocytose MP. The failure to remove mucus plugs can lead to bronchiectasis, bronchitis obliterans, and even acute respiratory failure and obstruction.[27]

MPP Treatment

When the pathogen could not be diagnosed early by peripheral blood sample or throat swab, the DNA or RNA pathogen in BALF was detected in time to identify the pathogen early. Once the diagnosis of MPP is confirmed, macrolides should be given promptly to prevent infection. If the treatment is ineffective or the disease progresses rapidly, the infection may be more complex. Macrolide resistant MPP infection or an overreaction, if necessary, the treatment should be adjusted to second-line anti-mycoplasma drugs (fleroxacin, tetracycline) or glucocorticoid drugs, and a bronchoscopy and BAL therapy should be scheduled as early as possible. Once the over-activation of NF-ƙB is observed, the signal transduction of NF-ƙB can be blocked by early administration;[11] examples include selective IKK inhibitors, proteasome inhibitors, KMOX (FK-506), IƙBα super inhibitors, glucocorticoids, and acetyl L-cysteine.

Limitations of the Study

This study was based on a single Grade III, Class A hospital child patient with SMPP. This implies that the results of this study may be biased when extrapolated to other populations. The study duration was short and the sample size was limited. We plan to expand the study population in future research to achieve a large multicenter population.

Conclusion

NF-ƙB is involved in the changes of inflammatory airways in children with SMPP. The higher the level of NF-ƙB in the airways, the more severe the clinical manifestations, and the longer the length of hospital stay will tend to be. Intracellular NF-ƙB is more closely related to sputum thrombus, pleural effusion, pericardial effusion, extrapulmonary complications, the higher the fever peak, the clinical manifestations, and days of hospital stay. We can use NF-ƙB as a detection index to support determining a patient's condition and prognosis, enabling the early implementation of relevant measures, select appropriate drugs to prevent the condition from becoming more serious, shorten the course of the disease, and reduce child mortality.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the First Affiliated Hospital of Xinxiang Medical University (Approval No.2021021). We obtained signed informed consent from the participants / legal guardians in this study.

Disclosure

The authors report no conflicts of interest in this work.

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