Int J Clin Exp Med 2020;13(2):691-699
www.ijcem.com /ISSN:1940-5901/IJCEM0103782

Original Article

The expression and significance of T lymphocyte subsets, interleukin-8, and tumor necrosis factor-α in children infected with streptococcus pneumoniae

Yanling Dai[1], Xiaomin Guo[2], Shuhua Pang[3], Chunyan Yang[4]

[1]Department of Laboratory, Chuxiong Medical and Pharmaceutical College, Chuxiong City, Yunnan Province, China; [2]School of Nursing, Chuxiong Medical and Pharmaceutical College, Chuxiong City, Yunnan Province, China; [3]Department of Clinical Laboratory, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China; [4]Department of Pharmacy, Chuxiong Medical and Pharmaceutical College, Chuxiong City, Yunnan Province, China

Received October 20, 2019; Accepted November 20, 2019; Epub February 15, 2020; Published February 28, 2020

Abstract: Objective: To investigate the expression and significance of T lymphocyte subsets, interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) in children infected with streptococcus pneumoniae (SP). Methods: Sixty children with SP infection were selected from our hospital as group A (GA). Sixty healthy children in the same period were categorized as group B (GB). Three mL of venous blood was obtained from the two groups after admission. Flow cytometry was adopted to test the proportions of $CD3^+$, $CD4^+$ and $CD8^+$ and the ratio of $CD4^+/CD8^+$. Enzyme-linked immunosorbent assay (ELISA) was used to determine the concentrations of serum IL-8 and TNF-α. ROC was introduced to evaluate the diagnostic value of serum IL-8 and TNF-α for SP. Results: The proportions of $CD3^+$, $CD4^+$ and $CD8^+$ and the ratio of $CD4^+/CD8^+$ in GA were significantly lower than those in GB ($P<0.05$). The concentrations of serum IL-8 and TNF-α in GA were significantly higher than those in GB ($P<0.05$). The sensitivity and specificity of diagnosis with IL-8 for SP were 86.67% and 78.33%, respectively. For diagnosis with TNF-α, the sensitivity and specificity were 73.33% and 90.00%, respectively. For diagnosis in combination with serum IL-8 and TNF-α, the sensitivity was 93.33% and the specificity was 91.67%. Conclusions: SP infection in children can cause immune dysfunction. Serum IL-8 and TNF-α are of important value for diagnosis of SP infection.

Keywords: Children, streptococcus pneumoniae, T lymphocyte subsets, IL-8, TNFα

Introduction

Streptococcus pneumoniae (SP) is the most common respiratory tract bacterial infection in children. It is the main cause of a series of infectious diseases clinically, such as septicemia, pneumonia, and meningitis, etc. [1]. According to the estimation of the World Health Organization (WHO), 1.7 million children (mostly from developing countries) die from SP infectious diseases each year. SP infection is one of the main causes of disability and death in children [2]. The appearance of SP is the result of the interaction between body and bacteria. The outcome of the disease depends mainly on the body's immune response to the bacteria [3]. Previous studies have shown that the body's immune response after SP infection will rapidly reach a peak. Death mostly occurs within 5 d after infection. It is related to multiple organ failure. Organ failure is caused by anti-inflammatory response imbalance and inflammatory mediators released by bacterial pro-inflammatory factors [4, 5]. Therefore, studying the changes in immune function and inflammatory response and possible pathogenesis is of great significance for the treatment and prognosis of SP in children.

SP infection in children can cause disorder of immune system. Furthermore, an inflammatory response occurs [6]. T lymphocyte subsets in the peripheral blood mainly include $CD3^+$, $CD4^+$ and $CD8^+$, etc. They mainly reflect the immune function status of the body, including immune regulation, defense and monitoring. Meanwhile, they also can be signs of infection, tumor and autoimmune diseases [7, 8]. As a chemokine, interleukin-8 (IL-8) is mainly secreted by epithelial cells and macrophages. It is chemotactic for

the neutrophils and can promote the inflammatory cells to induce chemotaxis and cell proliferation. Thus, the effect of cell damage and sterilization is achieved [9]. Tumor necrosis factor-α (TNF-α) is a kind of pro-inflammatory cytokine. In the initial research, TNF-α was reported to induce programmed cell death or apoptosis. This molecule is considered to be involved in many important cell regulation processes, such as proliferation, differentiation, growth and the immune response [10].

There are many studies on the effects of T lymphocyte subsets and inflammatory factors in pneumonia [11-14]. However, the specific role in children with SP infection is studied here. In this study, the levels of T lymphocyte subsets, IL-8 and TNF-α in the peripheral blood of children with SP infection were tested. Thus, the role of these measures in the occurrence and development of SP infection was investigated.

Materials and methods

General information

Sixty children with SP infection in our hospital were categorized as group A (GA), including 34 males and 26 females. They were aged 1 to 5 years and (3.1±1.1) years old in average. The course of the disease was 2-5 d and (3.2±1.3) days in average. The admission time was 1-4 days.

The inclusion criteria: The SP diagnostic criteria in Diagnosis and Management of Adult Community and Hospital Acquired Pneumonia issued by the National Institute for Health and Clinical Excellence (NICE) were met by the patients [15]. Infection by S. pneumoniae was diagnosed by the detection of a serological response against at least one of eight pneumococcal proteins (defined as an increase ≥2-fold in the IgG levels against Ply, CbpA, PspA1 and PspA2, PhtD, StkP-C, and PcsB-N, or an increase ≥1.5-fold against PcpA and isolation of streptococcus pneumoniae in blood cultures and/or pleural effusion [16]. Pneumonia manifestations (such as patchy shadow of both lungs or blurred lung texture, thickening disorder) were observed by X-ray chest examination in the patients. Patients aged 1-5 years old. The body temperature of patients is greater than 38.0°C. The patients present respiratory tract infection signs/symptoms (such as running nose, shortness of breath, cough, etc.).

The exclusion criteria: Patients who took immunosuppressive agents, pneumococcal vaccination, anti-inflammatory drugs during the past month or children with liver and kidney dysfunction, malignancy, congenital metabolic disease, endocrine systemic disease, autoimmune systemic disease, congenital heart disease, hemolytic jaundice, cerebrovascular disease, mental disorders or other acute conditions such as pulmonary edema, pulmonary embolism or patients had severe immunocompromised status with severe neutropenia (peripheral white blood cell (WBC) count <1,000/μl) [17] were excluded.

Sixty healthy children in the same time period were categorized as group B (GB), including 31 males and 29 females. They were aged 1 to 6 years. The average age was (3.2±1.3) years old. The children in GB had no respiratory tract infection, no chronic or acute disease, no immune or allergic disease within the last month. The guardian of each subject was given detailed explaination about the study and signed the informed consent form. This study was not contrary to ethics. The protocol was submitted to the Ethics Committee of the hospital for review. The study was implemented after the approval was obtained.

Test methods

Three mL of venous blood was taken from the subjects after admission. The blood was placed in an anticoagulant-free vacuum tube and anticoagulant tube containing potassium dipotassium ethylenediamine tetraacetate (EDTA-K2).

Test of T lymphocyte subsets in peripheral blood

DxFLEX flow cytometer (Beckman Coulter Commercial Enterprise (China) Co., Ltd.) was used to test the proportions of $CD3^+$, $CD4^+$ and $CD8^+$ and the ratio of $CD4^+/CD8^+$. One hundred μL of anticoagulative whole blood was placed in the TruCOUNT tube. Twenty μL of Fluorescein Isothiocyanate (FITC) labelled monoclonal antibody CD3-PC5, CD4-PE and CD8-ECD (Shanghai Hengfei Biotechnology Co., Ltd., China, Art. No.: IM2635U, 130-109-414, 737659) was added to each tube. After being mixed well, the resulting substance was placed at room temperature for 20 min. The cells were lysed with 500 μL of hemolysin. Then, they were placed at room temperature for 15 min. Five hundred μL

of PBS buffer was added. After being mixed well, the mixture was placed for 10 min at room temperature. The samples were tested on the flow cytometer. The proportions of $CD3^+$, $CD4^+$ and $CD8^+$ and the ratio of $CD4^+/CD8^+$ were read.

Determination of IL-8, TNF-α concentrations

Enzyme-linked immunosorbent assay (ELISA) was adopted to determine the concentrations of serum IL-8 and TNF-α. The test was performed with the reference to the instructions for use of IL-8, TNF-α ELISA (Shanghai Xinfan Biotechnology Co., Ltd., China, Art. No.: XF-HUMAN-1007, XF16189Q). The samples and kit were balanced for 30 min. The standard well, blank well and sample well were set. Fifty μL of standard and 50 μL of test sample were added to the standard well and sample well, respectively. Then, the addition of 50 μl of streptavidin-HRP was performed. The wells were covered with the closure plate membrane. Incubation was performed at 37°C for 60 min. The liquid was discarded and dried. Each well was given 200 μL of washing solution. After standing for 30 s, the washing solution was discarded. This step was repeated 5 times. Each well was mixed with 50 μl of developer A and B. After being mixed well, the resulting substance was incubated at 37°C for 10 min. Color development was conducted at room temperature in a dark place. Fifty μl of stop solution was added to each well. BIO-RAD680 (Beijing Tideradar Science and Technology Co., Ltd., China) was used to determine the OD value at 450 nm. The IL-8 and TNF-α concentrations were calculated.

Statistical methods

SPSS 22.0 (Easybio (Beijing) Science and Technology Co., Ltd.) was used for statistical analysis. The enumeration data were expressed with the number of subjects/percentage [n (%)]. Chi-squared test was adopted for comparison of enumeration data between the groups. The measurement data were expressed with mean ± standard deviation ($\bar{x}$ ± sd). Independent sample t test was introduced for comparison of measurement data between the groups. ROC was applied to evaluate the diagnostic value of serum IL-8 and TNF-α for SP. A logistic regression model was established with IL-8 and TNF-α as independent variables, and the area under the ROC curve of the joint diagnosis was fitted by the probability value in the model. $P<0.05$ implied a significant difference.

Results

General information

The gender, age, body mass index (BMI), head circumference, passive smoking, place of residence, mother's education, birth weight, feeding method, glutamic oxalacetic transaminase (AST), alanine transaminase (ALT), platelet (PLT) count, hemoglobin (Hb), glutamyl transpeptidase (GGT), white blood cell (WBC) count, C reaction protein (CRP), major pathogen and subdural effusion were not different between GA and GB ($P>0.05$) (**Table 1**).

Results of T lymphocyte subsets

The proportions of $CD3^+$, $CD4^+$ and $CD8^+$ in GA were respectively lower than those in GB ($P<0.05$). The ratio of $CD4^+/CD8^+$ in GB was higher than that in GA ($P<0.05$) (**Table 2**; **Figure 1**).

Results of serum IL-8 and TNF-α concentrations

The concentrations of serum IL-8 and TNF-α in GA were accordingly higher than those in GB ($P<0.05$) (**Table 3**; **Figure 2**).

Diagnostic value of serum IL-8 and TNF-α concentrations for SP

The ROC of serum IL-8 and TNF-α concentrations for diagnosis of SP were plotted. The AUC, cutoff value, sensitivity and specificity of serum IL-8 for diagnosis of SP were respectively 0.858 (95% CI: 0.787-0.928), 5.48 (pg/mL), 86.67% and 78.33%. The AUC, cutoff value, sensitivity and specificity of serum TNF-α were correspondingly 0.889 (95% CI: 0.834-0.944), 3.28 (pg/mL), 73.33% and 90.00%. Logistic regression model was established with IL-8 and TNF-α as independent variables. Logit (P combined diagnosis) = -15.365+1.138 IL-8+2.641 TNF-α, The ROC of serum IL-8 and TNF-α in combination for diagnosis of SP was further plotted. The AUC was 0.959 (95% CI: 0.925-0.993), cutoff value 0.51, sensitivity 93.33% and specificity 91.67% (**Tables 4** and **5**; **Figure 3**).

Table 1. General information of Group A and Group B [n (%)] ($\bar{x}\pm sd$)

Category	Group A (n = 60)	Group B (n = 60)	t/χ^2 value	*P* value
Gender			0.302	0.583
Male	34 (56.67)	31 (51.67)		
Female	26 (43.33)	29 (48.33)		
(Age)	3.1±1.1	3.2±1.3	0.455	0.650
BMI (kg/m^2)	15.21±1.35	15.24±1.18		
Head circumference (cm)	49.73±2.03	49.57±1.98	0.437	0.663
Passive smoking			1.046	0.306
Yes	11 (18.33)	7 (11.67)		
No	49 (81.67)	53 (88.33)		
Place of residence			0.657	0.418
City	45 (75.00)	41 (68.33)		
Rural	15 (25.00)	19 (31.67)		
Mother's education			0.853	0.837
Primary school	2 (3.33)	3 (5.00)		
Junior high school	6 (10.00)	4 (6.67)		
High school or secondary school	16 (26.67)	14 (23.33)		
The University	36 (60.00)	39 (65.00)		
Birth weight			0.209	0.648
Normal	3 (5.00)	2 (3.33)		
Low birth weight	57 (95.00)	58 (96.67)		
Feeding method			0.668	0.716
Breast milk	41 (68.33)	45 (75.00)		
Artificially	6 (10.00)	5 (8.33)		
Mixed feeding	13 (21.67)	10 (16.67)		
AST (U/L)	20.06±9.87	21.24±10.07	0.648	0.518
ALT (U/L)	25.63±11.58	24.67±12.29	0.440	0.660
PLT (10×10^9/L)	181.52±51.79	189.03±66.71	0.689	0.492
Hb (g/L)	138.09±35.67	132.85±40.62	0.751	0.454
GGT (U/L)	9.95±3.18	10.24±4.73	0.394	0.694
WBC (10×10^9/L)			0.144	0.705
>20.0	23 (38.33)	21 (35.00)		
≤20.0	37 (61.67)	39 (65.00)		
CRP (100 mg/L)			0.034	0.854
>100 mg/L	25 (41.67)	26 (43.33)		
≤100 mg/L	35 (58.33)	34 (56.67)		
Main pathogens			0.275	0.965
Klebsiella pneumoniae	6 (14.29)	5 (12.82)		
Staphylococcus aureus	4 (9.52)	3 (7.69)		
Streptococcus pneumoniae	18 (42.86)	16 (41.03)		
Escherichia coli	14 (33.33)	15 (38.46)		
Subdural effusion			0.543	0.461
Yes	28 (46.67)	24 (40.00)		
No	32 (53.33)	36 (60.00)		

Discussion

Streptococcus Pneumoniae (SP) infection is a common respiratory system disease in children. A series of complications can result. SP infection is a main factor leading to death in children. As a result, a great burden is placed on the family, life and society [18, 19]. With the

Table 2. Comparison of T lymphocyte subsets in peripheral blood of group A and group B ($\bar{x}$±sd)

Index	Group A (n = 60)	Group B (n = 60)	t value	*P* value
$CD3^+$ (%)	64.28±5.36	66.41±6.09	2.034	0.044
$CD4^+$ (%)	32.91±5.37	36.97±5.09	4.250	<0.001
$CD8^+$ (%)	24.58±6.34	27.05±6.18	2.161	0.033
$CD4^+/CD8^+$	1.08±0.47	1.43±0.54	3.787	<0.001

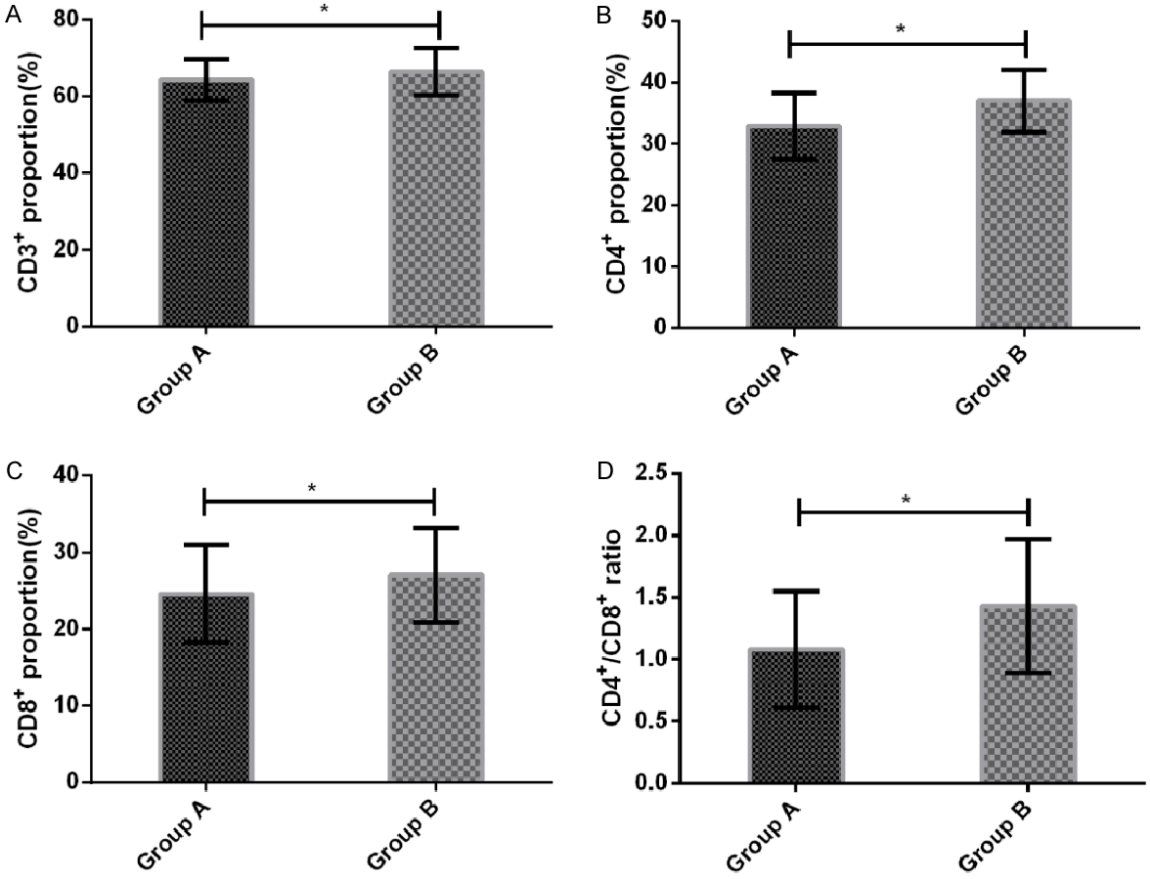

Figure 1. Comparison of T lymphocyte subsets in peripheral blood of group A and B. Comparison of $CD3^+$ proportions between group A and B (A); Comparison of $CD4^+$ proportions (B); Comparison of $CD8^+$ proportions (C); Comparison of $CD4^+/CD8^+$ ratio (D). Note: *implied P<0.05.

Table 3. Comparison of serum IL-8 and TNF-α concentrations in group A and group B ($\bar{x}$±sd)

Index	n	IL-8 (pg/mL)	TNF-α (pg/mL)
Group A	60	6.52±1.13	4.26±1.08
Group B	60	4.09±0.96	2.19±0.73
t value	-	12.690	12.300
P value	-	<0.001	<0.001

further understanding of the physiological and pathological mechanism of SP, the role of immune function and inflammatory reaction in the occurrence and development of SP has attracted much attention [20].

T lymphocyte subsets play an important role in human immunoregulation [21]. $CD3^+$ is the representative of the proportion of mature T lymphocytes. Its proportion is positively correlated with the immune function of T lymphocytes [22]. $CD4^+$ can stimulate B lymphocytes to produce antibodies. Its proportion is closely related to the level of lymphocyte factors [23]. $CD8^+$ is an inhibitory T cell. It can inhibit the antibody produced by B lymphocytes. The decrease in its

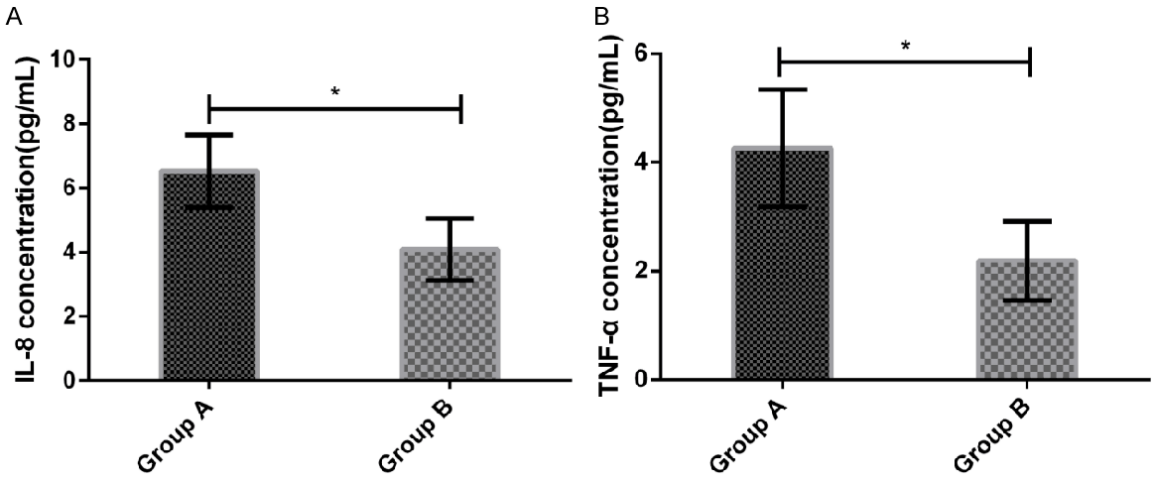

Figure 2. Comparison of serum IL-8 and TNF-α concentrations. Comparison of serum IL-8 concentrations (A); Comparison of serum TNF-α concentrations (B). Note: *implied P<0.05.

Table 4. Diagnostic value of serum IL-8 and TNF-α concentrations for SP

Diagnostic index	AUC	95% CI	Std. Error	Cut-off value	Sensitivity (%)	Specificity (%)
IL-8	0.858	0.787-0.928	0.036	5.48 (pg/mL)	86.67	78.33
TNF-α	0.889	0.834-0.944	0.028	3.28 (pg/mL)	73.33	90.00
IL-8+TNF-α	0.959	0.925-0.993	0.017	0.51	93.33	91.67

Table 5. Logistic regression analysis results of binomial classification

Variable	B	Std. Error	Wals	*P* value	OR	95% CI
IL-8	1.138	0.268	18.067	<0.001	3.121	1.847-5.276
TNF-α	2.641	0.606	19.024	<0.001	14.034	4.282-45.992
Constant	-15.365	3.060	25.211	<0.001	0.000	

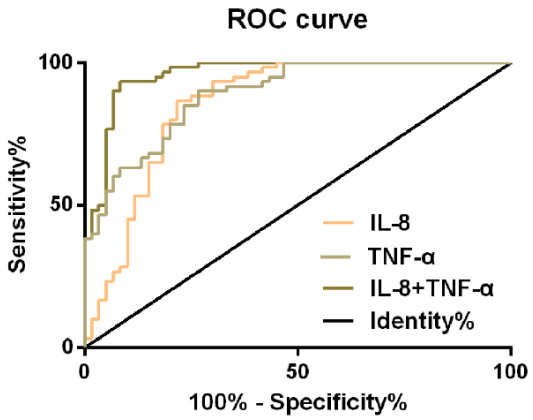

Figure 3. ROC of serum IL-8 and TNF-α concentrations for diagnosis of SP.

proportion indicates the decrease in immunosuppression ability [24]. $CD3^+$, $CD4^+$ and $CD8^+$ interact with each other in lymphocytes. $CD4^+$ and $CD8^+$ regulate and restrict each other. The imbalance of $CD4^+/CD8^+$ ratio indicates the abnormal immune function. $CD4^+/CD8^+$ ratio is closely related to the state of immunosuppression. A decrease in the ratio implies inhibition of the immune function [25, 26]. T lymphocytes play a certain role in the pathogenesis of SP. Previous studies have shown that T lymphocytes can increase significantly around the bronchus in the early stages of pneumonia. However, the specific effect of T lymphocytes in SP still needs to be further studied [27]. In this study, the proportions of $CD3^+$, $CD4^+$ and $CD8^+$ and the ratio of $CD4^+/CD8^+$ in GA were much lower than those in GB. The results showed that SP infection could lead to a decline of immune function and disorders of immune regulation function. In the report of Dockrell, et al [28], the number and activation of $CD4^+$ can affect the inflammatory cytokine network and the survival status of patients with SP. In the study of Weber, et al [29], $CD8^+$ cells play an important role in the regulation of pul-

monary neutrophil aggregation and the production of CXC chemokine. Therefore, it is necessary to understand the state of T lymphocyte subsets in SP patients. The clinical treatment of SP may be improved by regulating the immune response of children infected with SP.

IL-8 is a member of CXC chemokine and mainly secreted by epithelial cells. As a powerful chemokine of neutrophils, IL-8 is involved in the pathogenesis of acute and chronic pulmonary disease [30]. TNF-α is a protein. The protein is mainly produced by activated macrophages and monocytes. TNF-α participates in the normal inflammatory and immune responses. It is crucial for maintenance of balance in the body. The increase of TNF-α usually occurs under various pathological conditions, including sepsis, malignancy, heart failure and chronic infection [31]. There are many studies on the role of IL-8 and TNF-α in pneumonia. For example, the *in vitro* experiment by Chen, et al [32] has demonstrated that mycoplasma pneumoniae induces bronchial epithelial cells to produce IL-8 in a time-dependent manner. After stimulating IL-8, neutrophils produce myeloperoxidase and matrix metalloprotein. The concentration of neutrophils increase significantly. IL-8 may have great effect in the pathogenesis of mycoplasma pneumoniae. In the study of Bacci, et al [33], the levels of IL-8 and TNF-α are related to the early mortality of patients with community-acquired pneumonia. The higher the levels are, the worse the prognosis is. As a conclusion, IL-8 and TNF-α may play a vital part in the occurrence and development of pneumonia. However, there is little study on the specific changes of IL-8 and TNF-α in children with SP infection. The concentrations of serum IL-8 and TNF-α in GA were clearly higher than those in GB. The results showed that IL-8 and TNF-α might be involved in the occurrence and development of SP infection in children. In recent years, the onset mode, X-ray changes and clinical symptoms of SP are atypical due to the popularization and use of antibacterial drugs [34]. For this reason, the ROC of serum IL-8 and TNF-α concentrations for diagnosis of SP was plotted in this study. The results indicated that a test with IL-8 and TNF-α in combination had a better diagnostic value in children with SP infection. Therefore, IL-8 and TNF-α are expected to become good biological measures for diagnosis, treatment and prognosis of SP infection in children. The study of Li, et al [35] found that interleukin-2, interleukin-4, interleukin-10 and interferon-γ (IFN-γ) had certain diagnostic value for mycoplasma pneumoniae pneumonia (MPP). However, the sensitivity of TNF-α for diagnosis of MPP was only 59.3%. It was considered that mycoplasma pneumoniae and SP may be two different infections. Thus, this deviation was caused.

This study demonstrated that SP infection can cause immune dysfunction in children. The results showed the diagnostic value of IL-8 and TNF-α for SP. However, there are still some deficiencies in this study. First, longitudinal changes in T lymphocyte subsets and IL-8, TNF-α in children with SP infection were not observed. Second, the specific mechanism of T lymphocyte subsets and IL-8, TNF-α in the pathogenesis of SP was not studied in depth. Therefore, these deficiencies will be supplemented in future study. The results in this study will be further supported.

In summary, the SP infection in children can cause the immune dysfunction. Serum IL-8 and TNF-α have a good diagnostic value for SP infection.

Disclosure of conflict of interest

None.

Address correspondence to: Chunyan Yang, Department of Pharmacy, Chuxiong Medical and Pharmaceutical College, Chuxiong 675000, Yunnan Province, China. Tel: +86-0878-3875473; E-mail: nqvq1p5@163.com

References

[1] Schleicher GK, Hopley MJ and Feldman C. CD4 T-lymphocyte subset counts in HIV-seropositive patients during the course of community-acquired pneumonia caused by Streptococcus pneumoniae. Clin Microbiol Infect 2004; 10: 587-589.

[2] Geng Q, Zhang T, Ding Y, Tao Y, Lin Y, Wang Y, Black S and Zhao G. Molecular characterization and antimicrobial susceptibility of Streptococcus pneumoniae isolated from children hospitalized with respiratory infections in Suzhou, China. PLoS One 2014; 9: e93752.

[3] Sohail I, Ghosh S, Mukundan S, Zelewski S and Khan MN. Role of inflammatory risk factors in the pathogenesis of streptococcus pneumoniae. Front Immunol 2018; 9: 2275.

[4] Preston JA, Thorburn AN, Starkey MR, Beckett EL, Horvat JC, Wade MA, O'Sullivan BJ, Thomas R, Beagley KW, Gibson PG, Foster PS and Hansbro PM. Streptococcus pneumoniae infection suppresses allergic airways disease by inducing regulatory T-cells. Eur Respir J 2011; 37: 53-64.

[5] Calbo E and Garau J. Factors affecting the development of systemic inflammatory response syndrome in pneumococcal infections. Curr Opin Infect Dis 2011; 24: 241-247.

[6] Rodriguez AE, Bogart C, Gilbert CM, McCullers JA, Smith AM, Kanneganti TD and Lupfer CR. Enhanced IL-1beta production is mediated by a TLR2-MYD88-NLRP3 signaling axis during coinfection with influenza A virus and Streptococcus pneumoniae. PLoS One 2019; 14: e0212236.

[7] Lunde S, Kristoffersen EK, Sapkota D, Risa K, Dahl O, Bruland O, Mella O and Fluge O. Serum BAFF and APRIL levels, T-lymphocyte subsets, and immunoglobulins after B-cell depletion using the monoclonal anti-CD20 antibody rituximab in myalgic encephalopathy/chronic fatigue syndrome. PLoS One 2016; 11: e0161226.

[8] Pedrotti LP, Barrios BE, Maccio-Maretto L, Bento AF, Sena AA, Rodriguez-Galan MC, Calixto JB and Correa SG. Systemic IL-12 burst expands intestinal T-lymphocyte subsets bearing the alpha(4) beta(7) integrin in mice. Eur J Immunol 2016; 46: 70-80.

[9] Ungar B, Levy I, Yavne Y, Yavzori M, Picard O, Fudim E, Loebstein R, Chowers Y, Eliakim R, Kopylov U and Ben-Horin S. Optimizing anti-TNF-alpha therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. Clin Gastroenterol Hepatol 2016; 14: 550-557, e552.

[10] El-Tahan RR, Ghoneim AM and El-Mashad N. TNF-alpha gene polymorphisms and expression. Springerplus 2016; 5: 1508.

[11] Kusaka Y, Kajiwara C, Shimada S, Ishii Y, Miyazaki Y, Inase N, Standiford TJ and Tateda K. Potential role of Gr-1+ CD8+ T Lymphocytes as a source of interferon-gamma and M1/M2 polarization during the acute phase of murine legionella pneumophila pneumonia. J Innate Immun 2018; 10: 328-338.

[12] Wu HT, Zhou T, Liang X, Feng G, Lei Z, Wang ZY and Xian DJ. Curative effect of singulair combined with methylprednisolone in treatment of mycoplasma pneumonia and the effect on inflammatory response and peripheral blood lymphocyte subset content. 2017; 23.

[13] Li SQ and Pediatrics DO. Evaluation of the body's humoral immunity and peripheral blood T lymphocyte subset levels after mycoplasma pneumoniae infection. Journal of Hainan Medical University 2017; 23.

[14] Mei BJ. Effect of Tanreqing injection on mycoplasma pneumonia in patients of hs-CRP, PCT, T cell subsets. 2016.

[15] Rozenbaum MH, Pechlivanoglou P, van der Werf TS, Lo-Ten-Foe JR, Postma MJ and Hak E. The role of Streptococcus pneumoniae in community-acquired pneumonia among adults in Europe: a meta-analysis. Eur J Clin Microbiol Infect Dis 2013; 32: 305-316.

[16] Andrade DC, Borges IC, Vilas-Boas AL, Fontoura MSH, Araújo-Neto CA, Andrade SC, Brim RV, Meinke A, Barral A, Ruuskanen O, Käyhty H and Nascimento-Carvalho CM. Infection by Streptococcus pneumoniae in children with or without radiologically confirmed pneumonia. J Pediatr (Rio J) 2018; 94: 23-30.

[17] Watanabe H, Uruma T, Tazaki G, Kikuchi R, Tsuji T and Itoh M. Clinical factors associated with negative urinary antigen tests implemented for the diagnosis of community-acquired pneumococcal pneumonia in adult patients. Med Princ Pract 2015; 24: 189-194.

[18] Garin N and Marti C. Community-acquired pneumonia: the elusive quest for the best treatment strategy. J Thorac Dis 2016; 8: E571-E574.

[19] Silva-Costa C, Brito MJ, Pinho MD, Friaes A, Aguiar SI, Ramirez M and Melo-Cristino J, Portuguese group for the study of streptococcal I and portuguese study group of invasive pneumococcal disease of the pediatric infectious disease S. Pediatric complicated pneumonia caused by streptococcus pneumoniae Serotype 3 in 13-valent pneumococcal conjugate vaccinees, Portugal, 2010-2015. Emerg Infect Dis 2018; 24: 1307-1314.

[20] Baranek T, Morello E, Valayer A, Aimar RF, Brea D, Henry C, Besnard AG, Dalloneau E, Guillon A, Dequin PF, Narni-Mancinelli E, Vivier E, Laurent F, Wei Y, Paget C and Si-Tahar M. FHL2 regulates natural killer cell development and activation during streptococcus pneumoniae infection. Front Immunol 2017; 8: 123.

[21] Ding YM, Han LI, Medicine SO, University N and Pediatrics D. Effects of ribavirin combined with Clostridium butyricum powder on T lymphocyte subsets and inflammatory factors in children with rotavirus enteritis. 2017; 23.

[22] Martin NT, Black J, Aeffner F, Cerkovnik L and Krueger J. Abstract 4162: identifying T lymphocytes in IHC-stained tissues independently of CD3+ staining using morphometric features extracted by image analysis. Cancer Res 2016; 76: 4162-4162.

[23] Brandt D and Hedrich CM. TCRalphabeta(+) CD3(+)CD4(-)CD8(-) (double negative) T cells in autoimmunity. Autoimmun Rev 2018; 17: 422-430.

[24] Gao W, Kong M, Yao J, Wang F, Liu J, Wang D, Ma H, Ma L and Duan Z. Changes in prealbu-

min and body mass index associated with T lymphocyte subsets and nutritional status in chronic hepatitis B and HBV-cirrhosis patients. Clin Lab 2018; 64.

[25] Sommermeyer D, Hudecek M, Kosasih PL, Gogishvili T, Maloney DG, Turtle CJ and Riddell SR. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. Leukemia 2016; 30: 492-500.

[26] Laidlaw BJ, Craft JE and Kaech SM. The multifaceted role of CD4(+) T cells in CD8(+) T cell memory. Nat Rev Immunol 2016; 16: 102-111.

[27] Kadioglu A, De Filippo K, Bangert M, Fernandes VE, Richards L, Jones K, Andrew PW and Hogg N. The integrins Mac-1 and alpha4beta1 perform crucial roles in neutrophil and T cell recruitment to lungs during Streptococcus pneumoniae infection. J Immunol 2011; 186: 5907-5915.

[28] Dockrell DH, Whyte MKB and Mitchell TJ. Pneumococcal pneumonia: mechanisms of infection and resolution. Chest 2012; 142: 482-491.

[29] Weber SE, Tian H and Pirofski LA. CD8+ cells enhance resistance to pulmonary serotype 3 Streptococcus pneumoniae infection in mice. J Immunol 2011; 186: 432-442.

[30] Zou J, Zhou L, Hu C, Jing P, Guo X, Liu S, Lei Y, Yang S, Deng J and Zhang H. IL-8 and IP-10 expression from human bronchial epithelial cells BEAS-2B are promoted by Streptococcus pneumoniae endopeptidase O (PepO). BMC Microbiol 2017; 17: 187.

[31] Wang L, Chen Q, Shi C, Lv H, Xu X and Yu L. Changes of serum TNF-α, IL-5 and IgE levels in the patients of mycoplasma pneumonia infection with or without bronchial asthma. Int J Clin Exp Med 2015; 8: 3901.

[32] Chen Z, Shao X, Dou X, Zhang X, Wang Y, Zhu C, Hao C, Fan M, Ji W and Yan Y. Role of the mycoplasma pneumoniae/Interleukin-8/Neutrophil Axis in the pathogenesis of pneumonia. PLoS One 2016; 11: e0146377.

[33] Bacci MR, Leme RC, Zing NP, Murad N, Adami F, Hinnig PF, Feder D, Chagas AC and Fonseca FL. IL-6 and TNF-alpha serum levels are associated with early death in community-acquired pneumonia patients. Braz J Med Biol Res 2015; 48: 427-432.

[34] Seok H, Kang CI, Huh K, Cho SY, Ha YE, Chung DR and Peck KR. Risk factors for community-onset pneumonia caused by levofloxacin-non-susceptible streptococcus pneumoniae. Microb Drug Resist 2018; 24: 1412-1416.

[35] Li W, Liu YJ, Zhao XL, Shang SQ, Wu L, Ye Q and Xu H. Th1/Th2 Cytokine profile and its diagnostic value in mycoplasma pneumoniae pneumonia. Iran J Pediatr 2016; 26: e3807.