Am J Transl Res 2021;13(9):10517-10526
www.ajtr.org /ISSN:1943-8141/AJTR0128184

Original Article

The effects of the atomization inhalation of budesonide, salbutamol, and ipratropium bromide on the T-lymphocyte subset and inflammatory cytokine levels in children with asthmatic pneumonia

Yaqin Duan*, Huan Zhou*, Jianfeng Chen

*Department of Pediatrics, The People's Hospital of Huangpi, Wuhan 430300, Hubei Province, China. *Equal contributors and co-first authors.*

Received December 13, 2020; Accepted February 1, 2021; Epub September 15, 2021; Published September 30, 2021

Abstract: Objective: This study aimed to explore the effects of the atomization inhalation of budesonide (BUD), salbutamol (SAL), and ipratropium bromide (IB) on the T-lymphocyte subset and inflammatory cytokine levels in children with asthmatic pneumonia (AP). Methods: A total of 118 children with AP admitted to our hospital were selected as the study cohort and randomly divided into two groups. The study group, included 67 patients who were treated with the atomization inhalation of BUD, SAL, and IB. The control group, included 51 patients who were treated with the atomization inhalation of BUD. The two groups were compared in terms of their symptom disappearance times, the therapeutic effects, inflammatory cytokine changes, their pulmonary function indices [C-reactive protein (CRP), respiratory frequency, forced vital capacity (FVC), one-second forced expiratory volume (FEV1), blood oxygen saturation (SpO2)], and their T-lymphocyte subset levels before and after the treatment, and the incidences of adverse reactions after the treatment. Results: The symptom disappearance times in the study group were shorter than they were in the control group ($P<0.05$), and the overall response rate (ORR) was significantly higher in the study group ($P<0.05$). The IL-5, IL-6, and IL-10 levels were all lower in the study group ($P<0.05$), but the interferon-γ levels were higher in this group ($P<0.05$). The CRP level was lower in the study group ($P<0.05$), but the FVC, FEV1, and SpO2 levels were higher in this group ($P<0.05$). After the treatment, the $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels were all higher in the study group ($P<0.05$), but the $CD8^+$ level was lower in this group ($P<0.05$). The incidence of adverse reactions in the study group was lower than it was in the control group ($P=0.014$). Conclusion: The atomization inhalation of BUD, SAL, and IB is markedly effective in treating children with AP, and it can improve their immune function and reduce their inflammatory cytokines levels.

Keywords: Children with asthmatic pneumonia, T-lymphocyte subsets, inflammatory factor levels

Introduction

Pneumonia remains the number one killer of children globally. Although its mortality rate has been reduced with the development of medicine, its incidence is still high [1]. As a pulmonary inflammatory disease, pneumonia mainly affects the alveoli and is usually caused by viruses or bacteria [2]. Asthmatic pneumonia (AP), also known as bronchiolitis, is a common respiratory tract disease in pediatrics and is mostly induced by respiratory virus infections [3]. Its main clinical manifestations include coughing, wheezing, and choking, usually accompanied by fever [4]. With the constant changes in people's living habits, the incidence of AP in children has been rising yearly. Moreover, severe AP results in dyspnea and heart failure, and even threatens their lives [5, 6]. Traditionally, children with AP are mostly administered symptomatic treatments such as antivirals and antibiotics [7]. According to published data, viral infections cause the human body to produce a large number of cytokines and chemokines, thereby triggering the immune response [8]. Therefore, in addition to controlling the symptoms, improving the immune function is also crucial in the treatment of AP.

With the advances in research, we have found in many studies that budesonide (BUD), a kind of glucocorticoid, has satisfactory effects on

resisting inflammation and infection [9]. However, treatment with BUD is insufficient, and large doses of this drug lead to a series of complications and reduce the therapeutic effects [10]. As a short-acting β2-adrenergic receptor agonist, salbutamol (SAL) is usually used as an anti-asthmatic drug in combination with atomization to treat asthmatic bronchitis [11]. Ipratropium bromide (IB) is a bronchodilator used to treat symptoms such as bronchospasms and wheezing [12]. In addition, previous studies have pointed out that pneumonia can reduce the body's cellular immune function, and the main inflammatory factors that are mainly involved in the changes of cellular immune function include IL-5, IL-6, IL-12, interferon-γ, etc. [13], factors that have been found to be directly or indirectly involved in the regulation of various pathological and physiological responses in the body [14]. We have speculated that a combination of the three drugs has better antiviral and anti-infective effects on treating children with AP, and it can improve the inflammatory cytokines and enhance their immune functions. However, there is still a lack of relevant research data to support our conjecture. Therefore, the effects of the atomization inhalation of BUD, SAL, and IB on the T-lymphocyte subset and inflammatory cytokine levels in children with AP were explored in this study, in order to provide information for the future treatment of children with AP.

Data collection and therapeutic methods

Patient data

118 children with AP admitted to Panlong Hospital, Huangpi District People's Hospital from March 2018 to November 2019 were recruited as the study cohort and randomly divided into two groups. In the study group, 67 patients were treated with the atomization inhalation of BUD, SAL, and IB. In the control group, 51 patients were treated with the atomization inhalation of BUD. This experiment was approved by the ethics committee of our hospital, and the family members and the patients signed the informed consents.

Inclusion and exclusion criteria

Inclusion criteria: All the children included in the study were diagnosed with AP by laboratory and pediatric attending physicians in our hospital. All the children had complete case data. The family members of the children agreed to participate in this study. The children had complete clinical data and were able to undergo this treatment.

Exclusion criteria: Children allergic to the drugs used in this study. Children also suffering from other congenital or serious diseases. Children who could not undergo complete treatment for various reasons. Children with an abnormal coagulation function, a disturbance of consciousness, or mental disorders.

Therapeutic methods

After their admission, the children in both groups were given basic treatment such as routine cough suppressants, sputum excretions, and anti-inflammatories. In addition to this treatment, the children in the control group were treated with the atomization inhalation of BUD, 0.5 mg/time, twice/day. The oxygen flow was 4 L/min, and the atomization inhalations were controlled at 15-20 min/time. The children were treated for one week. In the study group, the children were treated with the atomization inhalation of BUD (0.5 mg/time), SAL (2 mg/time) and IB (0.15 mg/time) every day. The oxygen flow and the atomization inhalation times were the same as they were in the control group. The children were also treated for one week.

Outcome measures

The indicators were as follows: The symptom disappearance times (fever, cough, dyspnea, wheezing, moist rales), the therapeutic effects, changes in the inflammatory cytokine levels [IL-5, IL-6, IL-10, interferon-γ (IFN-γ)], the pulmonary function indices [C-reactive protein (CRP), respiratory frequency, forced vital capacity (FVC), one-second forced expiratory volume (FEV1), blood oxygen saturation (SpO2)], and the T-lymphocyte subset levels ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$, $CD8^+$) before and after the treatment, and the Incidence of adverse reactions after the treatment.

Statistical indicators

SPSS 24.0 and GraphPad 8 were used for the data analysis and figure plotting. The count

Table 1. General information [n (%)]

	Study group (n=67)	Control group (n=51)	t or X^2	P
Age (Years)	3.4±1.5	3.3±1.6	0.047	0.962
Gender			0.257	0.612
Male	36 (53.73)	25 (49.02)		
Female	31 (46.27)	26 (50.98)		
BMI (KG/cm^2)	13.32±2.15	13.16±2.25	0.393	0.695
Course of disease	6.12±2.11	6.36±2.34	0.584	0.561
Dwelling environment			0.053	0.819
City	46 (68.66)	48 (66.67)		
Countryside	21 (31.34)	25 (33.33)		
Parents' history of smoking			0.037	0.844
Yes	38 (56.72)	28 (54.90)		
No	29 (43.28)	23 (45.10)		
Parents' history of drinking			0.068	0.794
Yes	41 (61.19)	30 (58.82)		
No	26 (38.81)	21 (41.18)		
Body temperature °C	38.6±0.6	38.7±0.7	0.834	0.406
Nationality			0.083	0.773
Han	59 (88.06)	44 (86.27)		
Ethnic minorities	8 (11.94)	7 (13.73)		

data were expressed as (%) and compared between groups using chi-square tests. The measurement data were expressed as (mean ± standard deviation) and compared between groups using t-tests, one-way analyses of variance (ANOVA) and LSD post hoc tests were used for the comparisons between multiple groups, and repeated measures ANOVA and Bonferroni post hoc tests were used for the comparisons between multiple time points. When P<0.05, a difference was statistically significant.

Results

Clinical data

There were no significant differences between the study group and control group in terms of age, gender, body mass index (BMI), course of the disease, dwelling environment, parents' history of smoking, parents' history of drinking, body temperature, or nationality (P>0.05) (**Table 1**).

The symptom disappearance times

The fever, cough, dyspnea, wheezing, moist rales, and other symptom disappearance times were compared between the two groups. The results indicated that the fever (1.6±1.2), cough (3.1±1.1), dyspnea (2.1±0.7), wheezing (3.1±1.1) and moist rales (3.6±1.2) disappearance times in the study group were shorter than the corresponding times in the control group [(3.6±1.1), (5.6±1.4), (4.7±1.3), (5.3±1.6), (5.9±1.7)]. The differences were statistically significant (P<0.05) (**Figure 1**).

Therapeutic effects

The therapeutic effects on the children were compared between the two groups. In the study group, the cured, markedly effective, effective and ineffective rates were 28.36%, 37.31%, 28.36%, and 5.97%, respectively, and the overall response rate (ORR) was 94.03% in this group. In the control group, the four rates were 17.65%, 33.33%, 29.41% and 19.61%, respectively, and the ORR was 80.39% in this group. The ORR in the study group was significantly higher than it was in the control group (P<0.05) (**Table 2**).

Changes in the inflammatory cytokine levels before and after the treatment

Before and after the treatment, the changes in the inflammatory cytokine levels (IL-5, IL-6, IL-10, IFN-γ) were compared between the study group and the control group. Before the treatment, there were no significant differences in the four cytokine levels between the two groups (P>0.05). After the treatment, the IL-5, IL-6, and IL-10 levels were reduced, but the IFN-γ levels increased in both groups. The levels of the first three cytokines were all lower in the study group (P<0.05), but the IFN-γ levels were higher in this group (P<0.05) (**Figure 2**).

Changes in the pulmonary function before and after the treatment

Before and after the treatment, the changes in the pulmonary function indices (CRP, FVC, FEV1, SpO2) were compared between the study

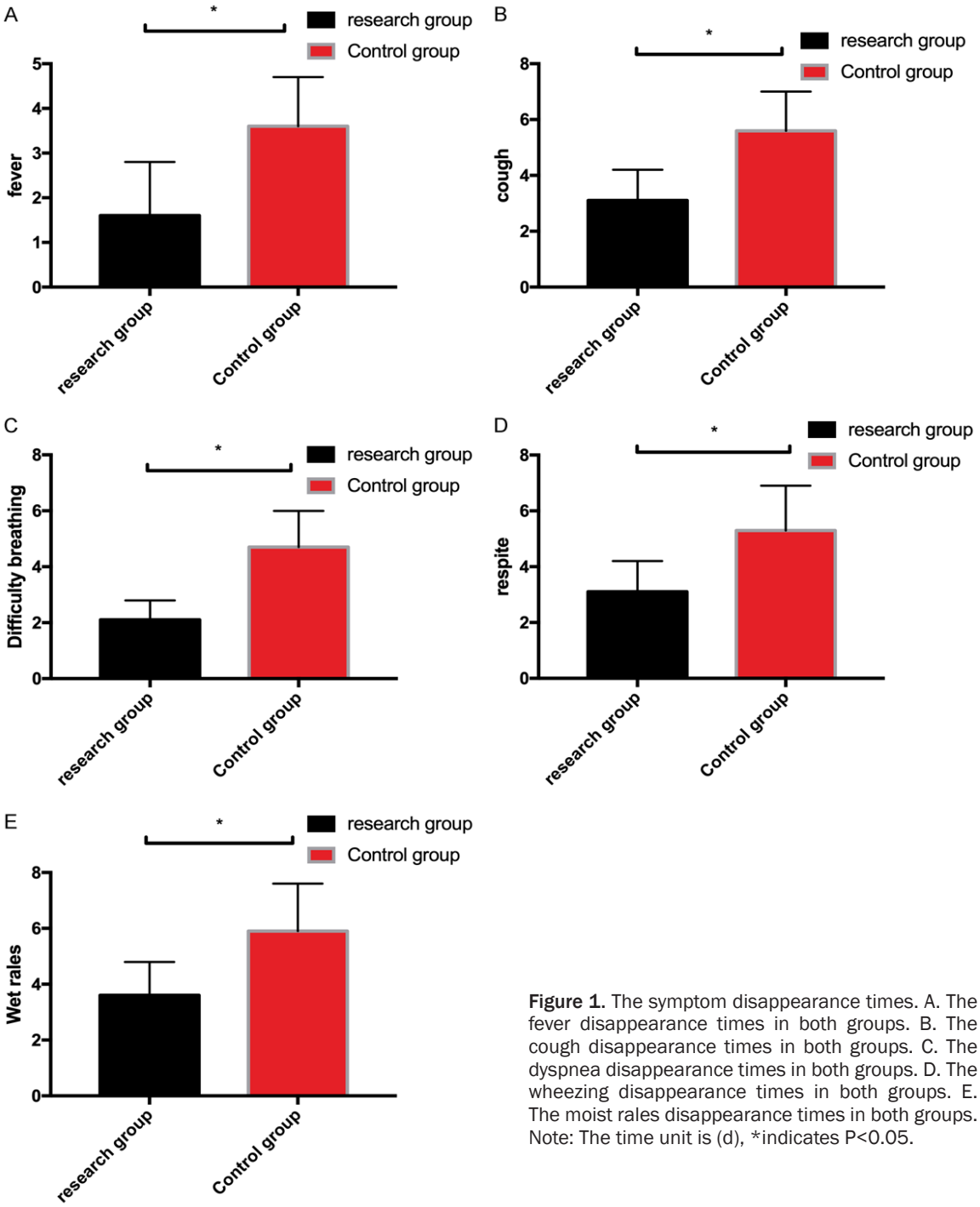

Figure 1. The symptom disappearance times. A. The fever disappearance times in both groups. B. The cough disappearance times in both groups. C. The dyspnea disappearance times in both groups. D. The wheezing disappearance times in both groups. E. The moist rales disappearance times in both groups. Note: The time unit is (d), *indicates P<0.05.

Table 2. Therapeutic effects

	Study group (n=67)	Control group (n=51)	X^2	P
Cured	19 (28.36)	9 (17.65)		
Markedly effective	25 (37.31)	17 (33.33)		
Effective	19 (28.36)	15 (29.41)		
Ineffective	4 (5.97)	10 (19.61)		
ORR (%)	63 (94.03)	41 (80.39)	5.150	0.023

group and the control group. Before the treatment, there were no significant differences in these indices between the two groups (P>0.05). After the treatment, the CRP levels were reduced, but the FVC, FEV1, and SpO2 levels increased in both groups. The CRP levels were lower in the study group (P<0.05), but the FVC, FEV1, and

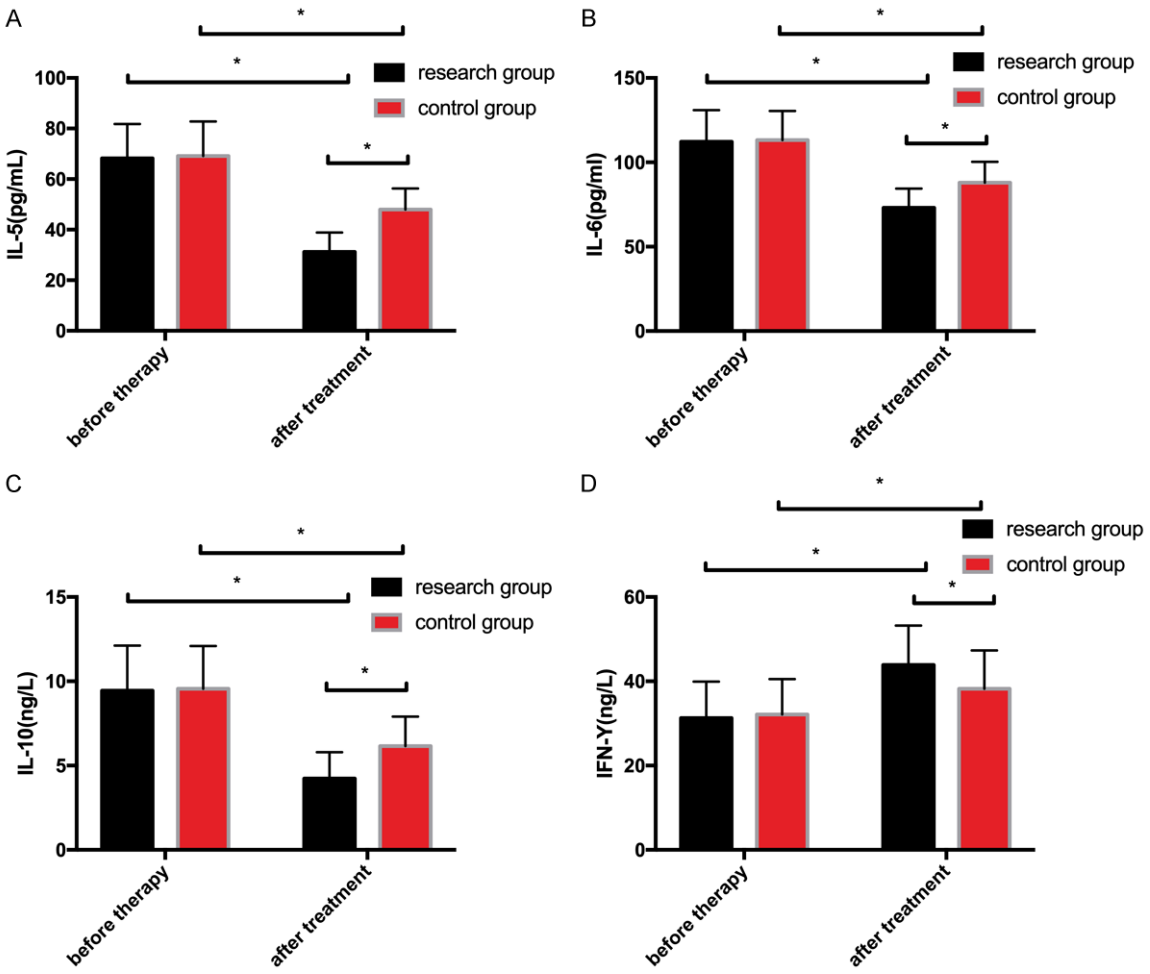

Figure 2. Changes in the inflammatory cytokine levels before and after the treatment. A. Changes in the IL-5 levels before and after the treatment. B. Changes in the IL-6 levels before and after the treatment. C. Changes in the IL-10 levels before and after the treatment. D. Changes in the IFN-γ levels before and after the treatment. Note: *indicates P<0.05.

SpO2 levels were higher in this group (P<0.05) (**Figure 3**).

Changes in the T-lymphocyte subset levels before and after the treatment

Before and after the treatment, the changes in the T-lymphocyte subset indicators ($CD3^+$, $CD4^+$, $CD4^+/CD8^+$, $CD8^+$) were compared between the study and control groups. Before the treatment, there were no significant differences in these indicators between the two groups (P>0.05). After the treatment, the $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels were increased, but the $CD8^+$ levels were reduced in both groups. The $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels were all significantly higher in the study group (P<0.05), but the $CD8^+$ levels were lower in this group (P<0.05) (**Figure 4**).

The incidence of adverse reactions after the treatment

After the treatment, the incidences of adverse reactions were compared between the two groups. The results showed that the adverse reactions in the study group were as follows: gastrointestinal reactions, 2.99%, headache, 1.49%, nausea, 1.49%, diarrhea, 1.49%, for a total of 7.46%. The adverse reactions in the control group were as follows: gastrointestinal reactions, 7.84%, headache, 5.88%, nausea, 3.92%, diarrhea, 5.88%, for a total of 23.53%. The total incidence of adverse reactions in the

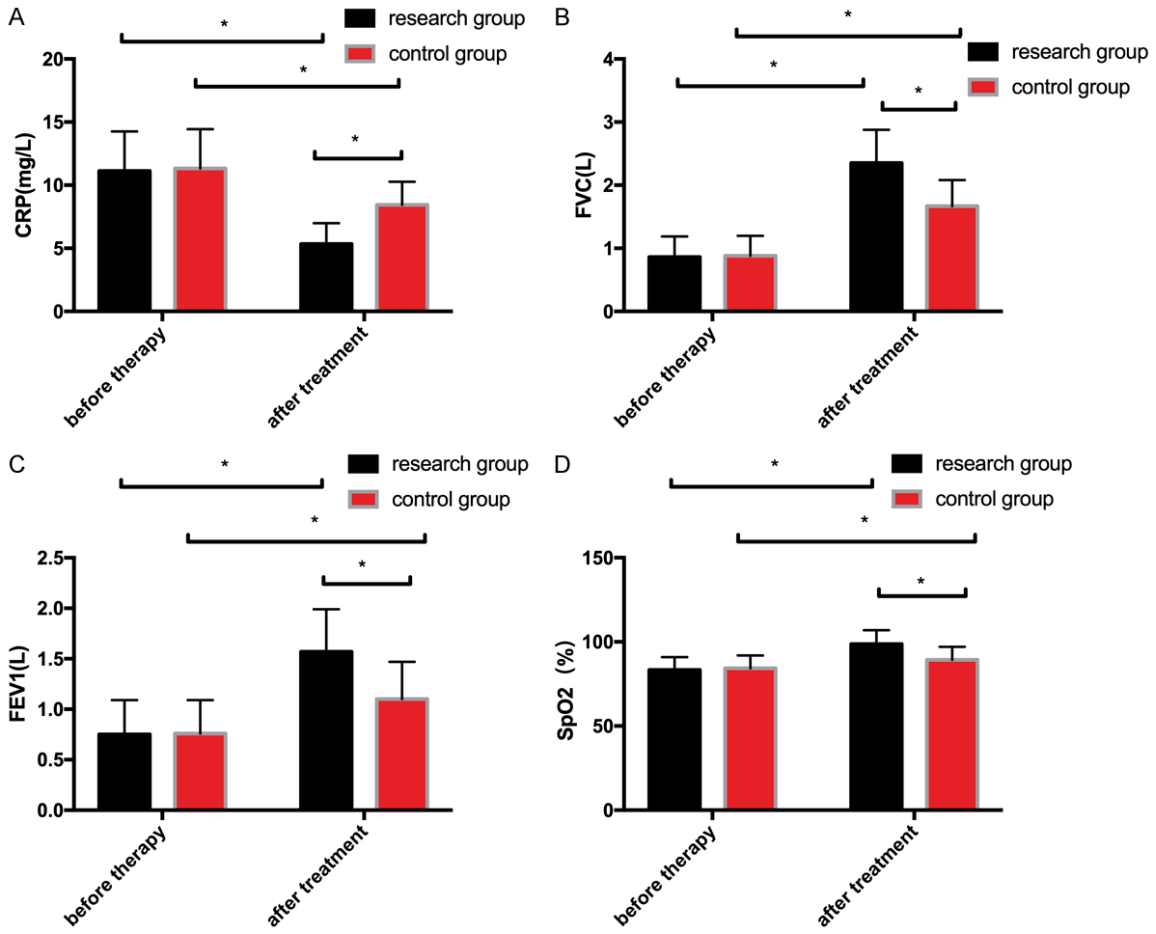

Figure 3. Changes in the pulmonary function before and after treatment. A. Changes in the CRP levels before and after the treatment. B. Changes in the FVC levels before and after the treatment. C. Changes in the FEV1 levels before and after the treatment. D. Changes in the SpO2 levels before and after the treatment. Note: *indicates P<0.05.

study group was significantly lower than the total incidence of adverse reactions in the control group (P=0.014) (**Table 3**).

Discussion

A common pediatric disease, AP is mainly caused by viral infections and is a serious threat to children's growth and their physical and mental health [15]. The traditional therapeutic method for the disease focuses on symptomatic treatment, which is ineffective and requires long-term treatment [16]. Moreover, children's bodies are not fully developed and their immune systems are not perfect, which results in an inability to prevent external viruses and infections, as well as an increased risk of viral infections [17]. Therefore, timely and effective treatment is essential for relieving the symptoms of children with AP. Therefore, the efficacy of the atomization inhalation of BUD, SAL, and IB in children with AP was evaluated in this study, so in order to confirm the therapeutic value of the combination in children. This is of great informational significance for the future clinical treatment of children with AP.

In our study, there were no significant differences between the two groups in terms of age, gender, BMI, course of the disease, dwelling environment, parents' history of smoking, parents' history of drinking, body temperature, or nationality. This suggests that follow-up research can be carried out. As for the symptom disappearance times of symptoms such as fever, cough, dyspnea, wheezing, and moist rales, the disappearance times in the study group were shorter than they were in the control group. This indicates that compared with a

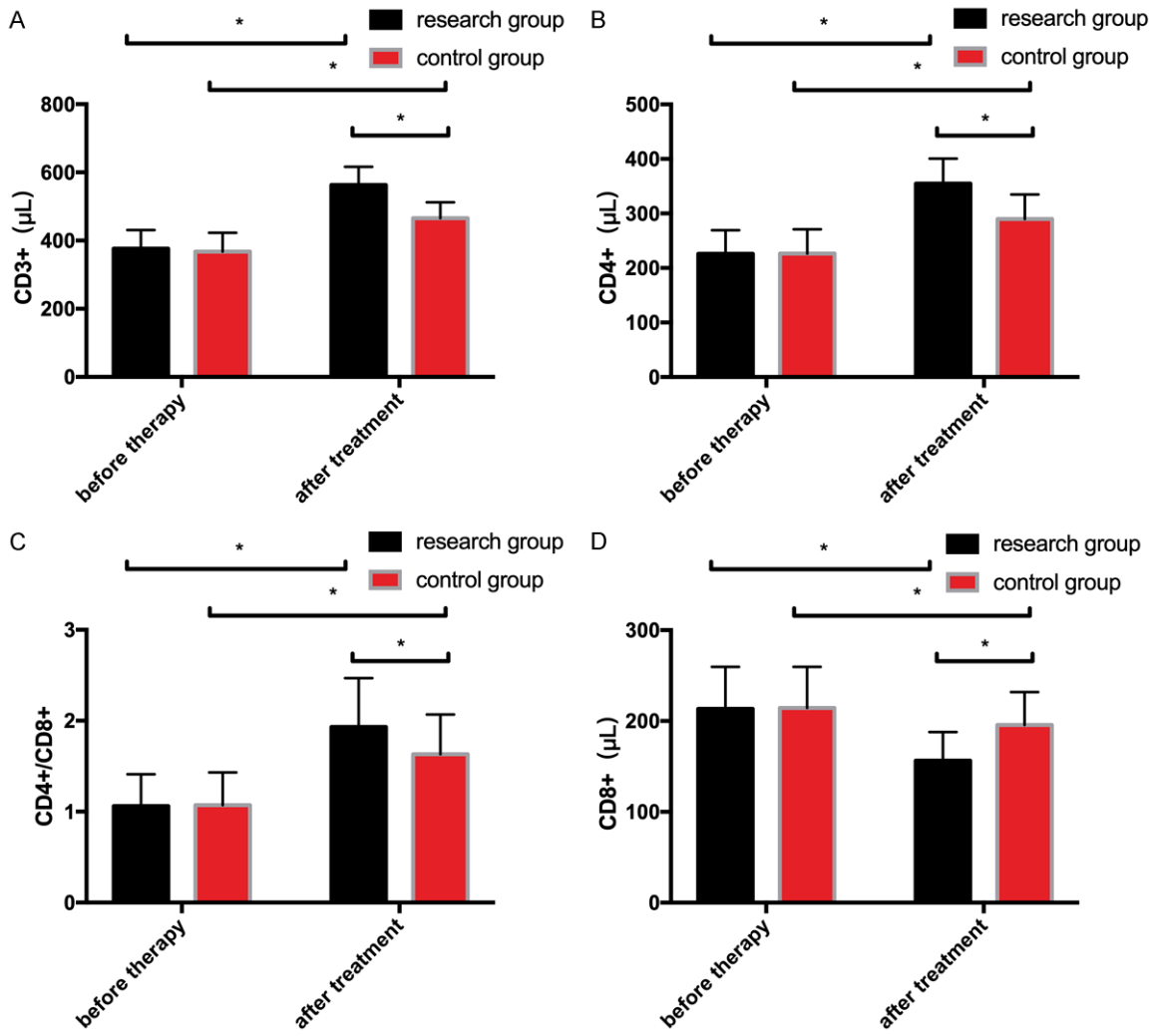

Figure 4. Changes in T-lymphocyte subset levels before and after the treatment. A. Changes in the $CD3^+$ levels before and after the treatment. B. Changes in the $CD4^+$ levels before and after the treatment. C. Changes in the $CD4^+$/$CD8^+$ levels before and after the treatment. D. Changes in the $CD8^+$ levels before and after the treatment. Note: *indicates $P<0.05$.

Table 3. The incidences of adverse reactions after the treatment

	Study group (n=67)	Control group (n=51)	X^2	P
Gastrointestinal reactions	2 (2.99)	4 (7.84)		
Headache	1 (1.49)	3 (5.88)		
Nausea	1 (1.49)	2 (3.92)		
Diarrhea	1 (1.49)	3 (5.88)		
Total	5 (7.46)	12 (23.53)	6.062	0.014

single medication, the atomization inhalation of the three drugs is more effective, and can promptly prevent AP from worsening and can help children recover. As for the therapeutic effects, the ORR in the study group was 94.03%, which was remarkably higher than the 80.39% in the control group. This further confirms the above conclusion and reflects the clinical effectiveness of the combination for children with AP. A glucocorticoid with highly effective local anti-inflammatory effects, BUD is usually combined with atomization to treat asthma, bronchitis, pneumonia, and other viral infectious diseases in children [18]. According to a large number of studies on this topic, this drug has satisfactory therapeu-

tic effects, but it also has some limitations. For instance, an excessive dosage of BUD alone increases the risk of complications, so its combination with other drugs has been gradually introduced to reduce its recommended dosage levels [19]. It has been shown that SAL, an antiasthmatic drug, can inhibit the release of allergenic substances such as histamines and prevent bronchospasms, thereby reducing airway inflammation and alleviating disease symptoms [20]. As reported by Bjermer and other researchers, SAL has a satisfactory effect on relieving the tension of airway muscles [21]. This is similar to our experimental results. IB, which is an anticholinergic drug, is markedly effective in relaxing the bronchial smooth muscles, preventing and treating asthma, and relieving wheezing symptoms [22]. We speculate that the synergy of the three drugs makes their therapeutic effects complementary, thus inhibiting the release of inflammatory mediators and achieving the best therapeutic effects on diseases. To verify the speculation, we compared the changes in the IL-5, IL-6, IL-10, and IFN-γ levels before and after the treatment between the two groups. After the treatment, the IL-5, IL-6, and IL-10 levels were reduced, but the IFN-γ levels increased in both groups The levels of the first three cytokines were all lower in the study group, but the IFN-γ levels were higher in this group. This shows that the combined treatment inhibits the inflammatory responses and alleviates the airway inflammatory responses, which supports our hypothesis. Next, we compared the changes in the CRP, FVC, FEV1, and SpO2 levels before and after the treatment between the two groups. After the treatment, the CRP levels were reduced but the FVC, FEV1, and SpO2 levels increased in both groups. The CRP level was lower in the study group, but the FVC, FEV1, and SpO2 levels were higher in this group. This demonstrates that the combined treatment not only relieves the children's symptoms quickly and effectively, it also relieves their lung function impairment and accelerates the recovery of their lung function. Therefore, it greatly helps to restore the children's signs. After that, we compared the $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ and $CD8^+$ changes before and after the treatment between the two groups. After the treatment, the $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels increased, but the $CD8^+$ levels were reduced in both groups. The $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels were all significantly higher in the study group, but the $CD8^+$ level was lower in this group. T-lymphocytes are important immune cells in the human body, and they play an important role in immune regulation [23]. $CD3^+$ T-lymphocytes can reflect the overall T cell levels and can be divided into $CD4^+$ and $CD8^+$ T lymphocytes according to the differences in the CD molecules on their surfaces. $CD4^+$ cells can activate the antigenic immune response state by secreting cytokines, and $CD8^+$ cells are inhibitory T-lymphocytes, that inhibit the function of $CD4^+$ T-lymphocytes and have cytotoxic effects [24]. Therefore, the $CD3^+$, $CD4^+$ and $CD4^+/CD8^+$ levels in the study group were significantly higher than they were in the control group, and the $CD8^+$ level was decreased. Viral infections in children aggravate their inflammatory responses, and their fragile immune functions (caused by incomplete development) can extremely easily result in a re-infection and aggravated conditions [25]. The experimental results of this study show that the combined treatment of drugs and the atomization inhalation are of great significance in improving the immune functions of children. At the same time, we have also speculated that such immune interventions may become another direction in the clinical treatment of AP, but this conjecture needs a detailed experimental analysis in follow-up research. Finally, we compared the incidences of adverse reactions after the treatment between the two groups. The total incidence of adverse reactions in the study group was 7.46%, which was significantly lower than the rate in the control group (23.53%). This further suggests that the combined treatment is safe for children with AP. However, there are still several limitations to this study. First, many other treatments are available for children with AP, so it is ruled out that the experimental results will be biased when they were treated with other combined therapies; moreover, the control was single, i.e., inhaled BUD. Second, the small cohort and short experimental period prevent us from analyzing the long-term prognosis. We will address these limitations with more experimental analyses to provide effective clinical guidance for the treatment of AP in children.

In summary, the atomization inhalation of BUD, SAL, and IB is markedly effective in treating children with AP, and it can improve their immune function and reduce their inflammatory cytokine levels.

Disclosure of conflict of interest

None.

Address correspondence to: Jianfeng Chen, Department of Pediatrics, The People's Hospital of Huangpi, No. 259 Baixiu Street, Qianchuan, Huangpi District, Wuhan 430300, Hubei Province, China. Tel: +86-13006346238; E-mail: CJF1122332020@163.com

References

[1] Mandell LA and Niederman MS. Aspiration pneumonia. N Engl J Med 2019; 380: 651-663.

[2] Koo HJ, Lim S, Choe J, Choi SH, Sung H and Do KH. Radiographic and CT features of viral pneumonia. Radiographics 2018; 38: 719-739.

[3] Romero-Espinoza JA, Moreno-Valencia Y, Coronel-Tellez RH, Castillejos-Lopez M, Hernandez A, Dominguez A, Miliar-Garcia A, Barbachano-Guerrero A, Perez-Padilla R, Alejandre-Garcia A and Vazquez-Perez JA. Virome and bacteriome characterization of children with pneumonia and asthma in Mexico City during winter seasons 2014 and 2015. PLoS One 2018; 13: e0192878.

[4] Li W, Ban C, Zhang J, Hu Y, Han B and Han B. Correlation study of cough variant asthma and mycoplasma pneumonia infection in children. Pak J Pharm Sci 2017; 30: 1099-1102.

[5] Castro-Rodriguez JA, Abarca K and Forno E. Asthma and the risk of invasive pneumococcal disease: a meta-analysis. Pediatrics 2020; 145: e20191200.

[6] Cazeiro C, Silva C, Mayer S, Mariany V, Wainwright CE and Zhang L. Inhaled corticosteroids and respiratory infections in children with asthma: a meta-analysis. Pediatrics 2017; 139: e20163271.

[7] Dankoff S, Li P, Shapiro AJ, Varshney T and Dubrovsky AS. Point of care lung ultrasound of children with acute asthma exacerbations in the pediatric ED. Am J Emerg Med 2017; 35: 615-622.

[8] Zaidi SR and Blakey JD. Why are people with asthma susceptible to pneumonia? A review of factors related to upper airway bacteria. Respirology 2019; 24: 423-430.

[9] Bateman ED, Reddel HK, O'Byrne PM, Barnes PJ, Zhong N, Keen C, Jorup C, Lamarca R, Siwek-Posluszna A and FitzGerald JM. As-needed budesonide-formoterol versus maintenance budesonide in mild asthma. N Engl J Med 2018; 378: 1877-1887.

[10] Bassler D, Shinwell ES, Hallman M, Jarreau PH, Plavka R, Carnielli V, Meisner C, Engel C, Koch A, Kreutzer K, van den Anker JN, Schwab M, Halliday HL and Poets CF; Neonatal European Study of Inhaled Steroids Trial Group. Long-term effects of inhaled budesonide for bronchopulmonary dysplasia. N Engl J Med 2018; 378: 148-157.

[11] Wang S, Liu F, Tan KS, Ser HL, Tan LT, Lee LH and Tan W. Effect of (R)-salbutamol on the switch of phenotype and metabolic pattern in LPS-induced macrophage cells. J Cell Mol Med 2020; 24: 722-736.

[12] Wang F, Zheng H, Zhang Y, Zhu H, Shi J, Luo Y, Zhang X, Mao H, Herth FJF and Luo F. Nebulized Ipratropium bromide protects against tracheal and bronchial secretion during bronchoscopy: a randomized controlled trial. Medicine (Baltimore) 2019; 98: e17942.

[13] Marsland AL, Walsh C, Lockwood K and John-Henderson NA. The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis. Brain Behav Immun 2017; 64: 208-219.

[14] Choi J, Moon S, Bae H, Kim YW, Lee D, Kim S, Seo Y, Wang HS, Choi YW, Lee MW, Ko JH, Lim I and Bang H. Alnus sibirica extracts suppress the expression of inflammatory cytokines induced by lipopolysaccharides, tumor necrosis factor-alpha, and interferon-gamma in human dermal fibroblasts. Molecules 2019; 24: 2883.

[15] Gao M, Wang K, Yang M, Meng F, Lu R, Zhuang H, Cheng G and Wang X. Transcriptome analysis of bronchoalveolar lavage fluid from children with mycoplasma pneumoniae pneumonia reveals natural killer and T cell-proliferation responses. Front Immunol 2018; 9: 1403.

[16] Peccini L, Pennoni S, Mencarini V, Saponara M, Palladino N, Principi N, Pennoni G and Esposito S. A peculiar case of pneumonia due to mycoplasma pneumoniae in a child with cystic fibrosis and sensibilization to aspergillus fumigatus. Pathogens 2019; 9: 15.

[17] Hancock DB and London SJ; CHARGE Pulmonary Function Working Group. Determinants of lung function, COPD, and asthma. N Engl J Med 2011; 364: 86-87.

[18] Kothe TB, Sadiq FH, Burleyson N, Williams HL, Anderson C and Hillman NH. Surfactant and budesonide for respiratory distress syndrome: an observational study. Pediatr Res 2020; 87: 940-945.

[19] Jorup C, Lythgoe D and Bisgaard H. Budesonide/formoterol maintenance and reliever therapy in adolescent patients with asthma. Eur Respir J 2018; 51: 1701688.

[20] Vet NJ, de Winter BCM, Koninckx M, Boeschoten SA, Boehmer ALM, Verhallen JT, Plötz FB, Vaessen-Verberne AA, van der Nagel BCH, Knibbe CAJ, Buysse CMP, de Wildt SN, Koch

BCP and de Hoog M. Population pharmacokinetics of intravenous salbutamol in children with refractory status asthmaticus. Clin Pharmacokinet 2020; 59: 257-264.
[21] Bjermer L, Abbott-Banner K and Newman K. Efficacy and safety of a first-in-class inhaled PDE3/4 inhibitor (ensifentrine) vs salbutamol in asthma. Pulm Pharmacol Ther 2019; 58: 101814.
[22] Murphy K, Mahmood N, Craven D, Gallagher J, Ross K, Speicher R, Rotta AT and Shein SL. Randomized pilot trial of ipratropium versus placebo in children with critical asthma. Pediatr Pulmonol 2020; 55: 3287-3292.
[23] Farhood B, Najafi M and Mortezaee K. CD8(+) cytotoxic T lymphocytes in cancer immunotherapy: a review. J Cell Physiol 2019; 234: 8509-8521.
[24] Gao M, Liu Y, Guo M, Wang Q, Wang Y, Fan J, Shen Y, Hou J, Wan Y and Zhu Z. Regulatory CD4(+) and CD8(+) T cells are negatively correlated with CD4(+) /CD8(+) T cell ratios in patients acutely infected with SARS-CoV-2. J Leukoc Biol 2021; 109: 91-97.
[25] Levine B, Mizushima N and Virgin HW. Autophagy in immunity and inflammation. Nature 2011; 469: 323-335.