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Original Article

Effects of ulinastatin injection combined with antibiotic therapy in children with severe pneumonia via levels of serums sB7-H3, CCL18 and GM-CSF

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Abstract: Objective: To probe and analyze the effects of ulinastatin injection combined with antibiotic escalation therapy in children with severe pneumonia by measuring the levels of serums sB7-H3, CCL18 and GM-CSF. Methods: A total of 106 children with severe pneumonia admitted to our hospital were randomized into the observation group (n=55) and the control group (n=51) based on a random number table. The control group was treated with antibiotic ladder-like therapy, while the observation group was treated with ulinastatin injection in addition to the antibiotic ladder-like therapy. We compared the therapeutic effects and the changes of serum inflammatory factors, sB7-H3, CCL18 and GM-CSF between the two groups. Results: The observation group showed higher overall effective rates than the control group (*P<0.05*). Both the time of antibiotics usage and the length of hospitalization of the children in the observation group were significantly shorter than the control group (*P<0.05*). After treatment, the levels of serum inflammatory factors of the children in the observation group were significantly lower than those of the control group (*P<0.05*). The levels of serum sB7-H3, CCL18 and GM-CSF in the children in the observation group were significantly lower than the control group (*P<0.05*). Conclusion: Treating severe pneumonia in children with ulinastatin injection combined with antibiotic ladder-like therapy can effectively improve the clinical efficacy of treatment, and its mechanism of action may be related to inhibiting the inflammatory response and regulating the immune balance in the human body. The therapy is highly safe and worthy of clinical application.

Keywords: Ulinastatin injection, antibiotic ladder-like therapy, children with severe pneumonia, sB7-H3, CCL18, GM-CSF

Introduction

Pneumonia is a common and frequently occurring disease in children, and it is also the leading cause of death in children. The previous definition of severe pneumonia was limited to whether children with pneumonia had symptoms and signs involving other systemic responses. Currently it is considered that children can be diagnosed with severe pneumonia as long as they have systemic inflammatory reactions and severe ventilation dysfunction [1]. The diagnosis of children with severe pneumonia covers their clinical symptoms, chest radiograph changes, etiological examination, and arterial blood gas analysis. Children with severe pneumonia are prone to systemic inflammatory response syndrome, so the treatment principles for severe pneumonia from the WHO include early hospitalization and early use of intravenous antibiotics [2]. However, the widespread use of antibiotics, especially spectral antibiotics, can often lead to the dysbacteriosis of children and the resistance of pathogenic bacteria, which makes a routine infectious diseases gradually evolve into severe pneumonia. If the children are not treated promptly and effectively, it can induce the failure of multiple organs and can lead to their death, which therefore poses a serious threat to the health and life safety of these patients [3].Proposed in the early 21st century, the idea of de-escalation treatment is to adopt the most appropriate broad-spectrum antibiotics in the initial stage

of the disease to effectively cover the pathogenic bacteria that may cause infection, such as Gram-positive bacteria and Gram-negative bacteria, so that the infection can be controlled; thereby adjusting the type of medicine and dosage according to the test results and the changes in the children's clinical symptoms after 2-3 days of medicine, and implement corresponding targeted treatment. De-escalation antibiotic therapy has now become an important guiding program for the treatment of severe pneumonia in children [4]. Ulinastatin injection is a kind of spectral protease inhibitor extracted from human urine, which has a variety of biological functions such as stabilizing lysosomal enzyme membranes, anti-inflammatory, anticoagulation and anti-oxidation effects [5]. In order to further enhance the clinical efficacy of treatment for children with severe pneumonia, this study probed and analyzed the effects of ulinastatin injection combined with antibiotics de-escalation therapy on children with severe pneumonia by measuring the levels of serum sB7-H3, CCL18 and GM-CSF. The report is as follows.

Materials and methods

Clinical data

A total of 106 children with severe pneumonia admitted to our hospital between March 2018 and March 2019 were selected as the research subjects, including 64 males and 42 females. The children were aged 2 to 14 years, with an average age of 6.28 (±2.10) years. Among them, there were 23 children with left lung disease, 27 with right lung disease and 56 with double lung disease. All the children were randomized into either the observation group (n=55) or the control group (n=51) based on a random number table. The study was approved by the Ethics Committee of our hospital.

Inclusion and exclusion criteria

Inclusion criteria: (1) The children conformed to the diagnostic criteria for severe pneumonia [6] and were accompanied by disturbance of consciousness, with arterial systolic blood pressure <90 mmHg and respiratory rate up to 30/min; some were complicated with septic shock and were confirmed by X-ray; (2) The children were aged 2 to 14 years old; (3) The parents of the children voluntarily signed the informed consent.

Exclusion criteria: (1) The children were complicated with diseases in other vital organs, such as heart, liver, and/or kidney; (2) The children were complicated with congenital heart disease and/or immune dysfunction disease; (3) The children had a history of complicated chronic bronchial asthma of the respiratory tract.

Methods

The children it the control group were treated with antibiotic de-escalation therapy. They received a continuous intravenous drip of Meropenem injection with a drug dose of 10~20 mg/kg every 8 hours. The patients then were given an intravenous infusion once every 12 hours after their condition was relieved. The sputum of the children before antibiotic treatment was inoculated in a blood plate, EMB plate or chocolate agar plate to isolate and culture the pathogenic bacteria. We then obtained the pathogenic examination result within 72 hours, developed the corresponding antibiotic de-escalation therapy program in accordance with the pathogenic culture results, and customized the corresponding high-sensitivity, narrow-spectrum, low-toxic and low-cost antibiotics for treatment.

The children in the observation group were treated with intravenous drip of Ulinastatin (UTI) injection in addition to the antibiotic de-escalation therapy of the control group (Guangdong Techpool Biochemical Medicine Co. LTD., G.Y.Z.Z. H20040506). Treatment consisted of 200,000 U UTI and 50 ml 0.9% NaCl injection for a micro-pump injection, twice per day, and 7 days was set as one course of treatment, and 2 consecutive courses were performed.

Evaluation criteria of clinical efficacy

Referring to the literature criteria [7], COMPLETE RESPONSE: The clinical symptoms and signs, such as consciousness disorders, fever and shortness of breath of the children, were completely improved or significantly reduced, and the pathological indicators of etiology and laboratory examination returned to normal, MARKED RESPONSE: The clinical symptoms and signs of the children were significantly improved, but the laboratory examination and etiological examination indexes did not completely returned to normal. RESPONSE: The

Table 1. Comparison of clinical data between the two groups of children

Group	Number of cases	Gender		Age (Age, ±s)	Lesion site		
		Male	Female		Left lung	Right lung	Two lungs
The observation group	55	34	21	6.13±1.97	13	11	31
The control group	51	30	21	6.34±2.35	10	16	25
t/X^2	-	0.099		0.500		-0.376	
P	-	0.753		0.618		0.707	

Table 2. Comparison of clinical efficacy between the two groups of children [n (%)]

Group	Number of cases	Complete response	Marked response	Response	No response	Overall response rate (%)
The observation group	55	23 (41.82)	15 (27.27)	12 (21.82)	5 (9.09)	90.91
The control group	51	17 (33.33)	9 (17.65)	8 (15.69)	12 (23.53)	76.47
x^2	-	-	-	-	-	4.097
P	-	-	-	-	-	0.043

clinical symptoms and signs of the children improved, and the laboratory examination indexes and etiological examination were improved to some extent. NO RESPONSE: Children did not achieve any of the improvements stated above. Overall response rate = Complete response rate + Marked response rate + Response rate.

Observational indexes

(1) The usage time of antibiotics and the hospitalization time between the two groups of children were compared. (2) The fasting venous blood of the two groups of children was taken in the morning before and after treatment. The serum was separated after centrifugation, and the levels of serum inflammatory factors of the two groups, including-α (TNF-α), interleukin-6 (IL-6), IL-8, Soluble B7-H3 (sB7-H3), granulocyte-macrophage colony stimulating factor (GM-CSF), and Chemokine ligands 18 (CCL18), were determined by enzyme-linked immunosorbent assay. The detection kit was purchased from Thermo Scientific Co, and was performed strictly in accordance with the operating instructions. The absorbance value at 492 nm was detected by a microplate reader. (3) The routine blood, urine, liver function and renal function tests were performed in the two groups of child patients before and after drug treatment, and the occurrence of adverse reactions of the two groups during drug treatment was observed and recorded.

Statistical analysis

The data was analyzed by statistical software SPSS 22.0; the measurement data conforming to a normal distribution was expressed by (x ± s), and *t*-test was used for comparison; the data were expressed by percentage, and the comparison was made by chi-squared test. *P<0.05* suggested that the difference was statistically significant.

Results

Clinical data

The clinical data between the two groups of children was not significantly different (*P>0.05*), as shown in **Table 1**.

Clinical efficacy

The overall response rate of the children in the observation group (90.91%) was significantly higher than that of the control group (76.47%), with a difference that was statistically significant (*P<0.05)*, as shown in **Table 2**.

Comparison of the usage time of antibiotics and the length of hospitalization between the two groups of children

The usage time of antibiotics and the length of hospitalization of the children in the observation group were significantly shorter than those of the control group (*P<0.05*), see **Table 3**.

Table 3. Comparison of the use time of antibiotics and hospitalization time between the two groups of children (d, ±s)

Group	Number of cases	Use time of antibiotics	Hospitalization time
The observation group	55	23.74±5.38	26.84±6.37
The control group	51	27.92±7.22	29.95±7.42
T	-	3.396	2.320
P	-	0.001	0.022

Table 4. Comparison of inflammatory factors before and after treatment between the two groups of children (±s)

Group	Time	TNF-α (ng/L)	IL-6 (μg/L)	IL-8 (μg/L)
The observation group (n=55)	Before treatment	83.19±14.28	215.73±45.22	0.31±0.09
	After treatment	35.64±9.37*	133.74±37.48*	0.16±0.05*
	t	20.647	10.353	10.805
	P	0.000	0.000	0.000
The control group (n=51)	Before treatment	81.96±15.74	220.85±51.36	0.33±0.10
	After treatment	49.83±10.36	164.27±41.28	0.19±0.07
	t	12.177	6.132	8.191
	P	0.000	0.000	0.000

Note: Comparison with the control group during the same period, *$P<0.05$.

Comparison of inflammatory factors before and after treatment between the two groups of children

The difference in levels of serum inflammatory factors TNF-α, IL-6 and IL-8 was not statistically significant between the two groups of child patients before treatment ($P>0.05$). After treatment, the levels of serum inflammatory factors of the two groups of children were significantly lower than those before treatment ($P<0.05$), and the levels of serum inflammatory factors in the children in the observation group were significantly lower than those in the control group ($P<0.05$), as shown in **Table 4**.

Comparison of the levels of serum sB7-H3, CCL18 and GM-CSF before and after treatment between the two groups of children

The difference in the levels of serum sB7-H3, CCL18 and GM-CSF were not statistically significant between the two groups of children before treatment ($P>0.05$). After treatment, the levels of serum sB7-H3, CCL18 and GM-CSF of the two groups of children were significantly lower than those before treatment ($P<0.05$), and the levels of serum sB7-H3, CCL18 and GM-CSF of the children in the observation group were significantly lower than those in the control group ($P<0.05$), as shown in **Table 5** and **Figures 1**-**3**.

Comparison of adverse reactions

The routine blood, urine, liver function and kidney function tests were performed in the two groups of children during the treatment, and no severe drug-related adverse reactions occurred among the patients (χ^2=0.000, P=1.000).

Discussion

As a common and frequently occurring disease seen in the clinic, severe pneumonia in children often has an acute onset, which results in serious conditions. Children with this disease often have multiple complications after onset. Therefore, early clinical treatment of severe pneumonia is particularly important, and it can help reduce the mortality rate of children and improve the clinical treatment effect [8]. At present, patients with severe pneumonia are mostly given antibiotics in clinic treatment. As there is a wide range of antibiotics which have different application scopes and effects, it has become a major focus of clinicians to correctly use antibiotics and combine them with other drugs to improve the clinical prognosis of children with severe pneumonia [9]. Studies have shown that [10, 11] antibiotic de-escalation therapy is one of the best methods to treat severe pneumonia at the early stage, which can quickly control the infection conditions of the

Table 5. Comparison of the levels of serums sB7-H3, CCL18 and GM-CSF before and after treatment between the two groups of children (±s)

Group	Time	sB7-H3 (μg/L)	CCL18 (ng/ml)	GM-CSF (μg/L)
The observation group (n=55)	Before treatment	9.84±2.16	81.27±13.42	1.27±0.38
	After treatment	6.17±1.59*	42.83±7.94*	0.52±0.15*
	t	10.148	18.283	13.615
	P	0.000	0.000	0.000
The control group (n=51)	Before treatment	10.08±2.54	83.21±15.44	1.31±0.26
	After treatment	7.40±1.67	59.38±10.37	0.74±0.22
	t	6.296	9.150	11.952
	P	0.000	0.000	0.000

Note: Comparison with the control group during the same period, *$P<0.05$.

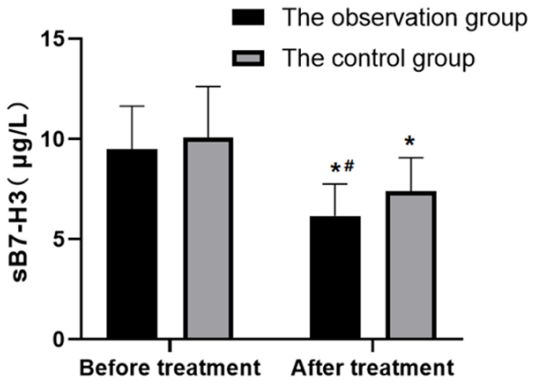

Figure 1. Comparison of serum sB7-H3 between two groups before and after treatment. (Note: Comparison with the same group before treatment, *$P<0.05$. Comparison with the control group in the same period, #$P<0.05$).

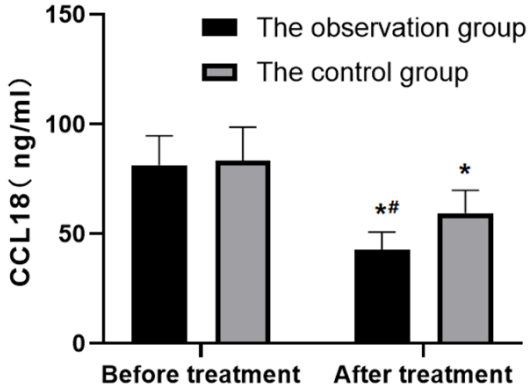

Figure 2. Comparison of serum CCL18 between two groups before and after treatment. (Note: Comparison with the same group before treatment, *$P<0.05$. Comparison with the control group in the same period, #$P<0.05$).

children and inhibit the activity of pathogenic bacteria to effectively control the disease condition. It has a significant effect on treating

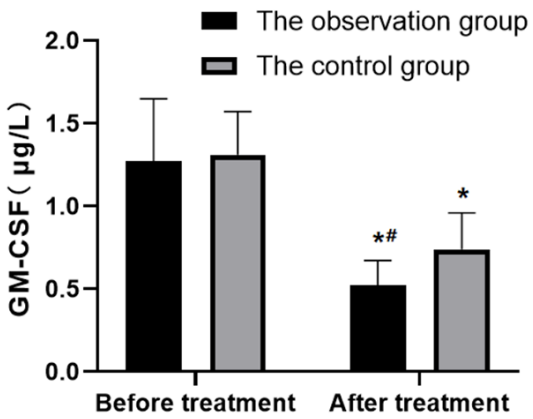

Figure 3. Comparison of serum GM-CSF between two groups before and after treatment. (Note: Comparison with the same group before treatment, *$P<0.05$. Comparison with the control group in the same period, #$P<0.05$).

severe pneumonia. Clinical practice has shown [12] that in the early stage of severe pneumonia treatment, broad-spectrum antimicrobials are the best choice for treatment. Antibacterial agents covering gram-positive bacteria and gram-negative bacteria can be selectively used to control and improve the condition of children and provide more valuable time for the analysis of etiology and the development of drug sensitivity experiments.

Ulinastatin injection is a spectral trypsin inhibitor purified from human urine which contains 143 isolated amino acids [13]. Studies have shown that [14, 15] UTI molecules have a loci that recognizes and binds to cell membrane receptors, and the negatively charged chondroitin sulfate sugar chain on the 10th serine enables its physiological function of stabilizing the cell membrane and lysosomal membrane; furthermore, UTI can inhibit the excessive acti-

vation of leukocytes and reduce the release of inflammatory factors and neutrophil elastase in the inflammatory cascade reaction. Pharmacological studies have shown that [16] UTI can significantly down-regulate the levels of TNF-α and IL-6 in sepsis induced in rats and reduce the lung injury caused by sepsis in rats. In addition, UTI can effectively inhibit coagulation factors Xa, XII and VIII, prevent prothrombin from being converted to thrombin, improve the microcirculation and tissue perfusion, and help improve systemic fibrinolysis abnormalities [17].

The results also showed that the clinical efficacy of the treatment for the children in the observation group was significantly superior to that of the control group, and both the usage time of antibiotics and length of hospitalization of the children in the observation group were significantly shorter than those of the control group. Using ulinastatin injection combined with antibiotic de-escalation therapy can effectively enhance the clinical efficacy of treatment in children with severe pneumonia and thus promote their rehabilitation. Meanwhile, the studies also indicate that the decreased degree of serum inflammatory factors TNF-α, IL-6 and IL-8 of the children in the observation group was significantly better than that of the control group. During the occurrence and development of severe pneumonia, neutrophils, macrophages and natural killer cells aggregate in the local mucosa of the respiratory tract due to chemotaxis. Bacterial lipopolysaccharide and endotoxins stimulate the above cells to release inflammatory mediators like TNF-α and IL-6, IL-8, endothelin, and histamine, causing congestion and edema of the pulmonary capillaries, thus resulting in diffuse inflammation of the pulmonary parenchyma or mesenchyme. This study suggests that ulinastatin combined with antibiotic de-escalation therapy can further strengthen the control of lung inflammation in children, thereby helping to block the disease progress, reduce the cascade caused by inflammation, and thus improve the clinical efficacy. Similar to results of relevant studies by other scholars [18], ulinastatin has a significant inflammatory control effect in the treatment of infectious diseases, which is related to the pharmacological effect that inhibiting the excessive activation of white blood cells.

The positive regulation effect of sB7-H3 on T cells is mainly to promote the proliferation and differentiation of CD4+ cells, and to promote the secretion of cytokines such as IFN-γ; its negative regulation effect is to inhibit the proliferation and differentiation of Th1 and Th2 cells, thereby participating in the immune regulation process in the body [19]. CCL18 is a chemokine highly expressed in the lungs and antigen-presenting cells, which acts as a chemotactic agent for lymphocytes and immature dendritic cells [20]. It can induce inflammatory cells such as lymphocytes and neutrophils to accumulate in inflammatory lesions and activate inflammatory signaling pathways to further expand the inflammatory response of the body and aggravate the degree of damage to the tissue [21]. GM-CSF is an inflammatory response sensitivity marker released by injured endothelial cells, which can promote the differentiation of hematopoietic progenitor cells into mononuclear macrophages, and can maintain the growth, reproduction and differentiation of mononuclear macrophages [22, 23]. Studies have shown that the increased levels of GM-CSF in children with pneumonia may be related to the imbalance of the proportion of T and B lymphocytes. The results of this study showed that after treatment, the levels of serum sB7-H3, CCL18 and GM-SF in the two groups of patients decreased, and the decrease in the children in the observation group was more obvious. This further suggests that ulinastatin injection combined with antibiotic de-escalation therapy may help control the inflammatory response in children and adjust the immune balance, which may be one of the mechanisms to enhance the efficacy of clinical treatment. At present, the influence of ulinastatin on immune function has been confirmed by scholars, but its specific mechanism of effect has not been completely elaborated, which is likely to be related to the inhibition of inflammatory response, improvement of local microcirculation, and promotion of body tissue repair. In addition, there was no drug-related adverse reactions that occurred during the treatment of the two groups of children, indicating the high safety of the treatment.

In this study, however, the sample size included was small and no in-depth research and analysis was conducted on the mechanism of action of drugs. Therefore, the sample size needs to

be expanded for further research, and in-depth research and analysis needs to be conducted on the specific mechanisms of action of drug treatment so that it can better guide clinical work.

In conclusion, treating severe pneumonia in children with ulinastatin injection combined with antibiotic de-escalation therapy can effectively improve the clinical efficacy of treatment in children, and its mechanism of action may be related to the inhibition of the inflammatory response and the regulation of immune balance. This therapy is highly safe and worthy of clinical popularization and application.

Disclosure of conflict of interest

None.

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