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Original Research Article

Effect of a combination of Xuebijing and thymosin α1 on patients with severe pneumonia complicated with sepsis, and its influence on serum inflammatory factors

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Abstract

Purpose: *To investigate the influence of a combination of Xuebijing and thymosin α1 on acute and septic bronchopneumonia (ASB) patients, and the effect of the treatment on inflammation.*
Methods: *Eighty-one (81) ASB patients admitted to Tianjin Medical University General Hospital, Tianjin, China from January 2015 to July 2019 were enrolled in the study. The patients were assigned to control group (n = 41) and a study group (n = 40). The control patients were treated with Xuebijing injection, i.v., while the study group received a combination of Xuebijing injection and thymosin α1 via the subcutaneous route. Patients' body temperature, heartbeat, white blood cell count, as well as other parameters, including blood gas indices, serum concentrations of IL-6, TNF-α and C-reactive protein (CRP), as well as well as blood gas indices were determined by enzyme-linked immunosorbent assay (ELISA). Bacterial clearance and therapeutic effectiveness were evaluated pre- and post-treatment.*
Results: *Post-therapy basal temperature, breathing, heartbeat, number of white blood cells and other indices decreased in study patients, relative to control ($p < 0.05$). Moreover, the levels of hydrogen ion concentration (pH) and arterial carbon dioxide partial pressure ($PaCO_2$) were significantly lower in study group than in control group ($p < 0.05$). Patients in the study group had lower serum levels of IL-6, TNF-α and CRP, when compared to control group, while bacterial load was significantly reduced in study subjects, relative to controls ($p < 0.05$).*
Conclusion: *Combined use of thymosin α1 and Xuebijing is significantly effective in the management of patients suffering from ASB. The combination therapy also improves the hemorheological status of patients. Thus, it is a potential therapeutic strategy for ASB.*

Keywords: *Acute bronchopneumonia, Xuebijing, Thymosin α1, Septicemia, Inflammation*

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INTRODUCTION

Septicemia results from infection-induced inflammatory reactions such as fever, leukopenia and reduced peripheral resistance, usually leading to low blood pressure (septic shock), organ malfunction and mortality. Indeed, septicemia accounts for approximately 30 % mortality [1]. Septicemia occurs mostly in the aged population. Thus, enhancements in the number of the aged have led to increases in cases of sepsis. In advanced nations, septicemia

affects 100 out of 100,000 people [2]. Septicemia is a severe response caused by systemic infection, with bronchopneumonia as a leading cause [3]. The etiology of sepsis starts with epitope transfer of antigen-manifesting cells to polymorphonuclear (PMN) leukocytes, macrophages, and T helper lymphocytes (Th), and then the cellular transcription factor NF-KB is activated and enters the nucleus where it forms a conjugate with DNA. Thereafter, the NF-KB-DNA complex triggers apoptosis and induces activation of Th lymphocytes to Thl cells, leading to up-regulations of inflammation-enhancing TNF-α, and IL-6 [4].

Xuebijing injection is a popular anti-sepsis polyherbal TCM containing *Angelica sinensis*, *Ligusticum wallichii*, red peony root, *Salvia* and safflower [5]. It has been reported that *Xuebijing* suppresses sepsis via mechanisms involving anti-inflammatory, antibacterial, endotoxin-neutralizing effects, thereby enhancing patients' survival [6]. Moreover, when used in combination with standard therapy, it reduces sepsis-related death in subjects with acute bronchopneumonia [7].

The immune-regulatory effects of thymosin α1, a protein hormone, have been reported in *in vivo* and *in vitro* studies, and it is an authorized antiviral and anti-cancer drug [8]. A study has shown that the combination of ulinastatin and *Xuebijing* produced more beneficial effect against septicemia than ulinastatin [9]. The aim of the present research was to investigate the effect of combination of *Xuebijing* and thymosin α1 in the treatment of sepsis.

METHODS

Background profiles of patients

Clinical information on 81 subjects with ASB on admission at *Tianjin Medical University General Hospital* from January 2015 to July 2019 were retrospectively analyzed. The subjects were assigned to 2 groups on the basis of treatment. The control group comprised 41 subjects (25 men and 16 women with mean age of 63.56 ± 5.44 years) treated with *Xuebijing* injection *i.v.* The study group had 40 subjects comprised of 26 men and 14 women (with mean age of 64.33 ± 5.32 years) who received a combination of *Xuebijing* injection and thymosin α1 via the subcutaneous route. The research received approval form the ethical authority of our institution (approval no. 20141112) in line with the principle of the Declaration of Helsinki [10]. Signed written informed permission was obtained from subjects and/or guardians.

The included patients were those who were diagnosed with ASB in line with guidelines for bronchopneumonia in adults in China [11], those who satisfied the 2016 international provisions on treatment of sepsis [12], and patients with intact clinical data. All subjects had impaired immune function or long-term use of ventilators, and they were severe pneumonia patients with long-term illness who often suffered from symptoms such as dyspnea, shortness of breath, asthma, and abnormal body temperature and pulse. The included patients undertook to give full cooperation during the study.

The excluded patients were those who used immunosuppressive drugs within the previous three months, subjects with acute kidney dysfunction and cancer, and those with psychiatric problems, communication problems, and illnesses likely to influence the outcome of the research.

Treatments

Following fasting, venous blood was collected from each patient. Serum was separated via centrifugation and preserved by freezing at -80 ^{0}C prior to analysis. Serum IL-6, TNF-α and CRP were assayed with ELISA kits. The IL-6 test kits were obtained from Shanghai Ultest Biotechnology Co. Ltd; kits for TNF-α were products of Shanghai Jingshen Bio-Eng. Co. Ltd. (model no. JK-(a)-2161), while the CRP test kits were bought from Shanghai Jingsheng Bio-Eng Co. Ltd (model no. JKSJ-1376). The determinations were carried out strictly in accordance with the kit instructions. The index of hydrogen ion concentration (pH) and partial pressure of arterial carbon dioxide ($PaCO_2$) were measured using ABL80 blood gas analyzer.

Both groups received effective anti-infection drugs. Broad-spectrum potent antibiotics such as carbapenem antibiotics (imipenem/cilastatin, meropenem and ertapenem) were applied. They also received supportive symptomatic treatment such as relieving cough and reducing sputum so as to mitigate asthma. If respiratory failure occurred, mechanical ventilation treatment was provided. Fluid therapy was given to supplement nutrients and hydration. In addition, they received other routine treatments such as regulation of BP and blood glucose, and maintenance of blood pH. Both groups were given *Xuebijing* (Tianjin CS Pharm. Co. Ltd.), 50 mg at a time, with 100 ml of physiological saline, *i.v.,* for about ½ h, thrice daily. The drug administration lasted for 10 days. In addition, patients in the study group received subcutaneous injection of thymosin α1 (Chengdu DJ Pharm. Co. Ltd) once daily at a

dose of 1.6 mg at a time. The treatment lasted for 10 days.

Evaluation of parameters/indices

General conditions

The vital signs before and after treatment were recorded and compared between the two groups.

Blood gas indices

A blood gas analyzer [model: ABL80 series; Radiometer Medical Equipment (Shanghai) Co., Ltd.] was used to determine the pH and $PaCO_2$ of patients before and after treatment, and statistical analysis was used to compare the values.

Levels of inflammatory factors

The serum levels of IL-6, TNF-α and CRP were assayed with ELISA. The IL-6 test kits were purchased from Shanghai Ultest Biological Technology Co. Ltd.; TNF-α kits were products of Shanghai Jingsheng Bioengineering Co. Ltd. (model no. JK-(a)-2161) and the CRP test kits were purchased from Shanghai Bio-Eng. Co. Ltd, (model no. JKSJ--1376). The determination was carried out strictly in accordance with the kit instructions. The levels of inflammatory factors were compared between the two groups.

Bacterial clearance

Bacterial clearance (BC) in both groups was monitored. The bacterial clearance (BC) was calculated as the sum of the no. of cleared cases, no. of hypothetical cases, and no. of replacement cases, divided by the total no. of patients, expressed as a percentage.

Treatment effectiveness

If respiration, body temperature, heartbeat and white blood cell count were normalized, the patients were deemed *cured*. If there was marked improvement in health, but one of these indexes was not normalized, the treatment was regarded as *markedly effective*. If there was marked improvement in the patient's condition, but these three indexes were not improved significantly, the treatment was deemed *effective*. However, if the patient's condition was unchanged or even aggravated, the treatment was regarded as *ineffective*. Total treatment effectiveness (TTE) was calculated using Eq 2.

$$TTE\ (\%) = \frac{(CR + ME + E)}{T} \times 100 \quad \text{..... (2)}$$

where *TTE* = total treatment effectiveness; *CR* = population of cured subjects; *ME* = population of markedly effective treatments; *E* = population of effective treatments; *T* = total population of patients.

Statistical analysis

Results were processed with SPSS 24.0 software, while graphics were generated using GraphPad Prism 7. Enumeration results are presented as percentage (%), and χ^2 test was used for 2-group comparison. All measured results are presented as mean ± SD, and *t*-test was employed for comparing data within a group. Values of $p < 0.05$ were considered as indicative of statistical significance.

RESULTS

Clinical profile of patients

Table 1 shows that baseline data were comparable in the two groups ($p > 0.05$).

Body temperature, respiration, heart rate, leukocytes before and after treatment

There were no significant differences in the levels of these indexes, including the number of leukocytes between the two groups prior to treatment. However, after treatment, these indexes, including the number of leukocytes in both groups were lower than those before treatment, with lower levels of indexes in the study group than in the control group ($p < 0.05$; Table 2).

Blood gas indices

Values of the blood gas indexes were comparable in the two groups before treatment. However, after treatment, there were reduced $PaCO_2$ and pH levels in both groups, with lower levels in the study group than in the control group. These results are shown in Table 3.

Blood levels of CRP, IL-6 and TNF-α

The levels of these factors were comparable in the two groups before treatment. However, post-treatment, there were decreased amounts of IL-6, TNF-α and CRP in both groups, relative to pre-treatment values, with lower levels in the study group than in the control group ($p < 0.05$). These data are shown in Table 4.

Table 1: Clinical data for patients in both groups

Variable	Study patients (40)	Control patients (41)	t (or F)	*P*-value
Sex			0.141	0.709
Male	26	25		
Female	14	16		
Age (years)	64.33±5.32	63.56±5.44	0.644	0.522
BMI (kg/m^2)	22.47±2.83	22.15±2.62	0.528	0.599
Place of residence			0.004	0.953
City	31	32		
Rural	9	9		
Nationality			0.863	0.353
Han	36	34		
Minority	4）	7		
Smoking history			0.102	0.749
Yes	29	31		
No	11	10		
Drinking history			0.106	0.745
Yes	21	23		
No	19	18		
Marital status			0.240	0.624
Married	37	39		
Unmarried	3	2		
Family history			0.103	0.748
Yes	18	17		
No	22	24		

Table 2: Comparison of pre- and post-treatment body temperature, breathing, heartbeat and white blood cell count between the 2 groups

Index		Study (n=40)	Control (n=41)	t	*P*-value
Body temperature (℃)	Before treatment	38.02±1.03	38.09±1.05	0.303	0.763
	After treatment	37.10±0.51*	37.52±0.59*	3.424	0.001
Respiration (times/min)	Before treatment	27.13±3.21	26.94±3.33	0.261	0.795
	After treatment	20.75±1.82*	23.65±2.03*	6.764	< 0.001
Heart rate (times/min)	Before treatment	104.83±13.21	105.04±16.81	0.062	0.950
	After treatment	75.86±11.69*	81.94±12.43*	2.267	0.026
Number of leukocytes (×10^9/L)	Before treatment	17.13±7.43	16.97±7.29	0.098	0.922
	After treatment	6.97±2.31*	10.54±3.64*	5.255	< 0.001

*P < 0.05, significant difference before and after treatment in the same group

Table 3: Comparison of blood gas indexes before and after treatment between the two groups

Index		Study group (n=40)	Control group (n=41)	*t*	*P*-value
pH	Before treatment	7.87±0.08	7.88±0.07	0.599	0.551
	After treatment	7.05±0.05	7.31±0.07	19.194	< 0.001
t		54.973	36.868		
P-value		< 0.001	< 0.001		
$PaCO_2$ (mm Hg)	Before treatment	54.29±5.24	53.98±5.21	0.267	0.790
	After treatment	35.18±5.29	39.23±5.33	3.488	0.001
t		16.232	12.672		
P-value		< 0.001	< 0.001		

Table 4: Comparison of serum IL-6, TNF-α and CRP levels before and after treatment between the 2 groups

Index		Study group (n=40)	Control group (n=41)	*t*	*P*-value
IL-6 (ng/L)	Before treatment	103.62±24.04	102.74±23.48	0.167	0.868
	After treatment	52.67±16.51	68.93±17.62	4.283	< 0.001
t		11.049	7.375		
P-value		< 0.001	< 0.001		
TNF-α (ng/L)	Before treatment	100.93±21.37	99.83±21.43	0.231	0.818
	After treatment	55.21±15.02	69.75±14.97	4.363	< 0.001
t		11.070	7.368		
P-value		<0.001	<0.001		
CRP (mg/L)	Before treatment	72.88±15.65	71.65±15.54	0.355	0.724
	After treatment	31.21±9.12	38.12±8.65	3.499	0.001
t		14.550	12.072		
P-value		< 0.001	< 0.001		

Table 5: Post-treatment bacterial clearance levels in the 2 groups

Bacterial clearance efficiency	Study group (n=40)	Control group (n=41)	*F*	*P*-value
Clearance	20	14		
Hypothetical clearance	11	9		
Replacement	4	5		
No clearanc	4	6		
Reinfection	1	7		
Clearance rate	87.50%	68.29%	4.322	0.038

Table 6: Effectiveness of treatments in the two groups

Group	Cure	Markedly effective	Effective	Ineffective	Total effectiveness (%)
Study (n = 40)	7	15	14	4	90.00
Control (n = 41)	4	12	13	12	70.73
F					3.742
P					0.029

Bacterial clearance

The results in Table 5 show that the bacterial clearance of the study group was 87.50 %, while that of the control group was 68.29 %.

Clinical effectiveness

The results presented in Table 6 show that total treatment effectiveness in study patients (90.0 %) was markedly superior to that in control patients (70.73 %).

DISCUSSION

An imbalance between levels of pro- and anti-inflammatory factors in subjects with acute bronchopneumonia eventually leads to sepsis. Sepsis causes impairment of immune function through reduction in the population of immune cells. It has been reported that the suppression of immunity in septicemia subjects is linked to lymphocytes [13]. *Xuebijing* is a polyherbal TCM that enhances blood flow and reduces blood stasis [14]. Research have demonstrated that *Xuebijing* and Thymosin α1 function as immune-regulatory and anti-inflammatory agents [15,16]. In a study, it was shown that thymosin α1 produced good effectiveness in septicemia patients [17]. As already stated, *Xuebijing* and thymosin α1 inhibit inflammation and regulate immune response. In the present research, there were lower body temperature, breathing rate, heartbeat and white blood cell population in study patients than in controls, post-treatment. Moreover, the study group had markedly superior total treatment effectiveness, relative to controls, indicating enhanced efficacy of the combined treatment. It has been reported the level of CRP, the most crucial index of inflammation and immune function, is higher in ASB subjects than in healthy controls [18].

The combined use of *Xuebijing* and thymosin α1 in this investigation resulted in markedly decreased concentrations of CRP, IL-6 and TNF-α. This demonstrates effective regulation of immune function and inflammation in the

patients. A similar result was obtained in a previous study which showed that *Xuebijing* markedly down-regulated TNF-α and IL-6 in a septicemic mouse model [19].

Prior to drug administration, blood rheology parameters were comparable in the study and control groups. However, blood rheology in the 2 groups were reduced, relative to the corresponding pre-treatment values, but were lower in study subjects than in control subjects. Thus, the combined treatment was efficacious in mitigating bronchopneumonia symptoms of patients and normalizing blood gas and pro-inflammatory factors in patients, without undesirable drug-related adverse events. This is because *Xuebijing* inhibits the assemblage platelets, accelerates blood circulation, increases vascular tone, improves oxygen levels, removes enzymes catalyzing bradykinin generation such as hydrolase and protease, repairs gaps in cells lining the blood vessels, reduces vascular porosity, and reduces levels of pro-inflammatory mediators [20]. Thymosin α1 stimulates the presentation of antigens on macrophage receptors, thereby enhancing antigen neutralization potential and significantly improving immunity and preventing inflammatory response in septicemic subjects.

Limitations of the study

This study has some shortcomings. It used a small sample size, and it lacked effective statistical analysis based on big data due to limited experimental conditions. Besides, results demonstrated that thymosin α1 reduced death in sepsis subjects, but a follow-up after intervention was not conducted in this study. Therefore, in subsequent studies using a larger sample size, the patients will be followed up and investigated to constantly improve the experiments and obtain more validated research results.

CONCLUSION

Thymosin α1, in combination with *Xuebijing* injection, significantly ameliorates ASB, improves blood gas pH and $PaCO_2$, eliminates septicemia and bacteria, and lowers the concentrations of serum pro-inflammatory factors. Therefore, the combination treatment is a potential therapeutic strategy for ASB.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yan Li and Jie Cao conceived and designed the study, and drafted the manuscript. Yan Li, Suhang Wang, Jing Zhang and Jie Cao collected, analyzed and interpreted the experimental data. Yan Li and Suhang Wang revised the manuscript for important intellectual contents. All authors read and approved the final manuscript.

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