THE CORRELATION BETWEEN SERUM CD_4^+ CD_{25}^+ TREG CELLS, HMGB1, LAC, AND ENDOTOXIN LEVELS AND THE SEVERITY OF SEVERE PNEUMONIA COMPLICATED WITH SEPSIS

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ABSTRACT

Objective*: To investigate how the levels of CD_4^+ CD_{25}^+ regulatory T lymphocytes (CD_4^+ CD_{25}^+ Treg), high mobility group protein B1 (HMGB1), blood lactate (Lac) and endotoxin (ET) correlate with the severity of severe pneumonia with sepsis.*

Methods: *68 patients with severe pneumonia and sepsis admitted to our hospital from March 2018 to June 2019 were randomly selected as the study sample, and 68 healthy subjects who had physical examinations in our hospital at the same time were selected as the control group. According to their prognoses, the patients were divided into a survival group (51 cases) and a death group (17 cases). The clinical data, Acute Physiology and Chronic Health Evaluation (APACHE II) score, serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET levels were compared. Pearson correlation test was used to analyze the correlation between serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET levels and the severity of severe pneumonia with sepsis.*

Results: *The respiratory rate, heart rate, body temperature and leukocyte count in the study group were significantly higher than those in the control group, and the platelet count was significantly lower than that in the control group ($P<0.01$). Compared with the control group, the levels of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac, ET and APACHE II in the study group were significantly higher ($P<0.01$). Compared with the survival group, the levels of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac, ET and APACHE II in the dead group were significantly higher ($P<0.01$). According to Pearson correlation analysis, the levels of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET in serum were positively correlated with APACHE II score, which means they were positively correlated with the severity of severe pneumonia complicated with sepsis ($P<0.05$).*

Conclusion: *The levels of serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET are significantly correlated with the severity of severe pneumonia complicated with sepsis, which can be used as an important index to judge the severity of illness and the prognosis of patients.*

Keywords: *CD_4^+ CD_{25}^+ treg cells, HMGB1, lac, endotoxin, severe pneumonia with sepsis, severity, correlation.*

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Introduction

Sepsis is a kind of systemic inflammatory reaction induced by infection factors. Its occurrence and development are closely related to abnormal immune response. According to relevant statistics, the mortality rate caused by sepsis and multiple organ failure in ICU is more than 60%. Even in the United States, where patients benefit from the world's most advanced medicine, more than 800,000 patients contract sepsis every year. Despite active treatment, the mortality rate is still increasing year-upon-year[(1)].

It has been established that sepsis is not an independent disease, but a result of the combined action of multiple systems including the immune system[(2)]. Pneumonia refers to inflammation involving the airway, alveoli and pulmonary interstitium. Common pneumonia can be cured after active and effective treatment, but if the pathogenic factors continue to exist, they will aggravate the condition of pneumonia and it may even develop into severe pneumonia[(3)]. Severe pneumonia is a common se-

rious and even fatal pulmonary infectious disease, which can lead to sepsis and respiratory failure. Sepsis is the most common lung involvement; it causes respiratory failure, which is the main cause of death in patients with severe pneumonia.

Sepsis and severe pneumonia often interact. Because of the aggravation of clinical drug abuse, most patients will have serious drug resistance, which makes clinical treatment more difficult. If the pathogenic factors cannot be removed in time, the patient's condition will further deteriorate, even causing death. Therefore, early recognition, early diagnosis and early treatment are of great significance to judge the severity and prognosis of a patient's condition and to reduce patient mortality[4].

In this study, serum CD_4^+ CD_{25}^+ regulatory T lymphocytes (CD_4^+ CD_{25}^+ Treg), high mobility group protein B1 (HMGB1), and blood Lactic acid (Lac) were measured, and endotoxin (ET) levels were monitored, and the correlation between the above indexes and the severity of severe pneumonia with sepsis was investigated.

Materials and methods

General information

68 patients with severe pneumonia and sepsis who were treated in our hospital from March 2018 to June 2019 were randomly selected as the study sample.

The inclusion criteria were as follows:

• All patients met the diagnosis and treatment criteria for sepsis formulated by the Chinese Medical Association[5], and all patients met the clinical diagnosis and treatment criteria for severe pneumonia formulated by the Chinese Medical Association[6];

• Each patient's body temperature was above 38 oC or below 36 oC, accompanied by inflammatory reactions such as leukocytosis;

• The study was approved by the ethics committee of our hospital, and all of them met the medical ethics;

• All patients' medical records were complete, and they were able to cooperate with the study;

• All patients and their family members agreed to participate in the study and signed a written agreement.

The exclusion criteria were as follows:

• Patients younger than 20, or older than 85;

• Patients who had a history of special treatment, such as surgery, radiotherapy or chemotherapy, within one month before participating in the study;

• Patients with serious liver and kidney function and/or heart function insufficiency;

• Patients with infectious diseases;

• Patients with known abnormal blood or coagulation function;

• Patients with immune dysfunction. 68 healthy subjects, who underwent a physical examination in our hospital at the same time, were selected as the control group.

Observation indicators

General information: the age, sex, body mass index (BMI), smoking history, respiratory rate, heart rate, body temperature, white blood cell count, platelet count and other clinical data were compared for all subjects.

5 mL of fasting elbow median venous blood was taken from each subject in the morning after admission. The blood was left to stand, and serum was separated from blood cells at a speed of 3000 r/min using a low-temperature high-speed centrifuge (Hunan Hennuo Instrument Equipment Co., Ltd., model: MiniStar 10K) The supernatant was put it in a -80°C ultra-low temperature refrigerator (Shanghai Precision Instrument Co., Ltd., model: DW-40L328), and kept frozen for inspection.

The Acute Physiology and Chronic Health Evaluation (APACHE II) score was used to judge the severity of the disease in patients in the study sample. The APACHE II score is composed of an acute physiology score (APS), an age score (Age), and a chronic health score (CPS). The APS score is expressed using a scale of 0 to 60 points, the Age score on a scale of 0 to 6 points, the CPS score on a scale of 2 to 5 points, and the total APACHE II score is expressed using a scale of 0 to 71 points.

The higher the score, the more serious is the patient's condition.The levels of CD_4^+ CD_{25}^+ Treg cells were measured by flow cytometry (Miltenfa Biotech, Germany, model: MACSQuant 10). The changes of Lac levels in patients were measured (Shanghai Hanfei Medical Instrument Co., Ltd., model: Rapidlab348).

Patients were divided into two groups: a survival group (51 cases) and a death group (17 cases), according to their prognosis. The changes of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac, and ET were compared between the two groups of patients. The correlation between serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET levels and the severity of severe pneumonia complicated with sepsis was analyzed by Pearson correlation test.

Statistical methods

χ^2 was used to compare the counting data in this study, which was expressed in [n%]. The measurement data were compared by independent sample t-test, expressed in (x±s). The APACHE II score was used to judge the severity of the condition of patients in the study group.

The levels of CD_4^+ CD_{25}^+ Treg cells in the two groups were measured by flow cytometry, the levels of HMGB1 and ET in serum were measured by enzyme-linked immunosorbent assay, and the changes of Lac levels in the two groups were measured by an automatic blood gas analyzer.

Pearson correlation test was used to analyze the correlation between serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET levels and the severity of severe pneumonia with sepsis. In this study, spss22.0 software was used for statistical data analysis, and the statistical results (P<0.05) were regarded as statistically significant differences.

Results

Changes in general clinical data of the two groups

There was no significant difference in general clinical data, such as age, gender, BMI, and smoking history, between the study sample and the control group (P>0.05). The average respiratory rate, heart rate, body temperature, and white blood cell count of the study sample were significantly higher than the average of those of the control group. The average platelet count was significantly lower than the control group (P<0.01). See Table 1.

Group	Study sample (n = 68)	Control group (n = 68)	χ^2/t	*P*
Age (years old)	66.21±9.15	64.53±11.96	0.920	0.359
Gender (cases, %)				
Male	42 (61.76)	43 (63.24)	0.031	0.859
Female	26 (38.24)	25 (36.76)		
BMI (kg/m^2)	23.46 ±1.72	24.18±2.56	1.925	0.056
Smoking history (cases, %)	34 (50.00)	30 (44.12)	0.472	0.492
Breathing frequency (times/min)	27.38±2.34	17.27±1.62	29.293	<0.001
Heart rate (beats/min)	103.47±10.42	73.41±9.31	17.740	<0.001
Body temperature (°C)	39.21±0.85	37.54±1.45	8.193	<0.001
White blood cell count ($\times10^9$/L)	16.87±6.68	8.66±2.54	9.473	<0.001
Platelet count ($\times10^9$/L)	92.41±28.40	283.66±22.54	43.497	<0.001

Table 1: Changes in general clinical data of the two groups.

Comparison of serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET levels and APACHE II scores between patients in the study sample and the control group

Compared with the control group, the levels of serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac, and ET and APACHE II scores in the study sample were significantly increased (P<0.01). See Table 2.

Group	Cases (n)	CD_4^+ CD_{25}^+ Treg cells (%)	HMGB1 (ng/mL)
Study sample	68	3.73±0.23	116.84±47.97
Control group	68	0.38±0.54	43.79±17.17
t		47.006	5.170
P		<0.001	<0.001

Group	Cases (n)	Lac (mmol/L)	ET (pg/mL)	APACHE II scores (points)
Study sample	68	6.55±1.02	15.41±2.38	19.28±3.64
Control group	68	0.87±0.27	4.15±0.63	6.84±2.11
t		44.391	37.715	24.382
P		<0.001	<0.001	<0.001

Table 2: Comparison of serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET levels and APACHE II scores between patients in the study group and the control group (x±s).

Comparison of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac, and ET levels in patients with different prognosis

Compared with the survival group, serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac, and ET levels and APACHE II scores in the death group were significantly increased (P<0.01). See Table 3.

Group	Cases (n)	CD_4^+ CD_{25}^+ Treg cells (%)	HMGB1 (ng/mL)
Survival group	51	2.56±0.26	82.68±24.62
Death group	17	5.67±1.57	147.97±31.79
t		16.115	13.390
P		<0.001	<0.001

Group	Cases (n)	Lac (mmol/L)	ET (pg/mL)	APACHE II scores (points)
Survival group	51	4.26±0.78	7.62±1.04	14.62±3.88
Death group	17	8.84±1.14	23.39±3.01	29.14±3.18
t		27.342	40.835	23.868
P		<0.001	<0.001	<0.001

Table 3: Comparison of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac, and ET levels in patients with different prognosis (x±s).

Correlation analysis of serum CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET levels with the severity of severe pneumonia with sepsis

According to Pearson correlation analysis, the levels of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET in serum were positively correlated with APACHE II

score; that is, they were positively correlated with the severity of severe pneumonia with sepsis (P<0.05). See Table 4.

Indicators	APACHE II scores	
	r	*P*
CD_4^+ CD_{25}^+ Treg cells	0.828	<0.05
HMGB1	0.420	<0.05
Lac	0.915	<0.05
ET	0.908	<0.05

Table 4: Correlation analysis.

Discussion

Pneumonia refers to the inflammation of the terminal airway, alveoli and pulmonary interstitium caused by physical and chemical factors, pathogenic microorganisms, immune damage, stress, or allergy. Severe pneumonia is a common serious and even fatal pulmonary infectious disease. Most patients have mixed infection, which causes diseases that are difficult to control. If further treatment is not actively sought, the infection can have a serious impact on the health of patients, even endangering life[7]. Severe pneumonia mainly originates from the lungs, but it can often cause systemic infection, leading to serious and even fatal infectious diseases. Severe pneumonia is often associated not only with serious lung injury but also with sepsis and other diseases, and is accompanied by systemic inflammatory response syndrome or multiple organ failure, usually with rapid development[8]. Therefore, it is important to establish the correlation between the severity of severe pneumonia and sepsis.

Immune dysfunction plays an important role in the occurrence and development of sepsis. Treg cells are a subset of T cells concerned with immunoregulation, which plays an important role in the immune system of the body, with negative regulation as its main feature[9]. Treg cells play an important regulatory role in the immune disorder of sepsis, because it is mainly manifested as an immunosuppressive effect, and this can significantly reduce the damage caused by excessive inflammatory response to the body, especially in the early cellular immune response of sepsis[10]. According to relevant reports, the percentage of Treg cells in the peripheral blood of patients with sepsis is significantly higher than that of the healthy control group. The level of CD_4^+ CD_{25}^+ Treg cells in the study sample was significantly higher than that in the control group[11]. This finding suggests that the degree of inflammatory response and immune function of patients with severe pneumonia and sepsis are in a state of mutual restriction.

HMGB1 is a non-histone protein in the nucleus that can participate in the proliferation, differentiation, and migration of tumor cells, promote the growth of nerve cells, and affect the function of vascular endothelial cells. In addition, HMGB1 is an important inflammatory mediator and inflammatory cytokine. HMGB1 in cells can enter the extracellular through active secretion of activated cells and passive release of necrotic cells, thus participating in the cell inflammatory response[12]. It has been found that HMGB1 is a potential "late" inflammatory factor[13], which generally occurs within 16-24 hours after ET stimulation, significantly later than the production of other, early, inflammatory factors. ET is formed by the action of the cell wall of Gram-negative bacteria. When ET is synthesized with the cell wall of lipopolysaccharide bacteria, it will be transported to the cell surface in the cell wall structure and become the component of the cell wall outer membrane. According to reports, when human cells are affected by ET, the hypothalamic thermoregulatory center will be stimulated to produce the Schwarzman reaction, affect white blood cells, activate complement, reduce fever, reduce blood pressure, and other phenomena[14]. Some scholars have found that ET is the main trigger of sepsis, and about 50% of sepsis shock is caused by ET. If the body is seriously infected with Gram-negative bacilli and this infection is not controlled in time, ET will accumulate in the blood, resulting in high levels of ET in the body[15]. Lac is the final product of glucose anaerobic fermentation, mainly produced in bone, muscle, brain and red blood cells. When the tissue perfusion is reduced, the coagulation system is damaged, or microthrombosis causes microcirculation disturbance, this also causes the hypoxia of tissue cells and the activation of anaerobic digestion pathway, resulting in a large amount of Lac. It has been found that due to the lack of perfusion or arterial oxygen supply in patients with severe infection, glucose anaerobic fermentation will increase, and the level of Lac, a metabolite, will increase significantly. The increase of Lac level is an early sensitive biochemical index of tissue perfusion and oxygen delivery deficiency[16].

In this study, the results showed that the levels of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET in the study sample were significantly higher than those in the control group. Levels in the death group were significantly higher than those in the survival group, and they were positively correlated with the

APACHE II score, which played an important role in evaluating the severity and prognosis of patients.

In conclusion, the levels of CD_4^+ CD_{25}^+ Treg cells, HMGB1, Lac and ET in serum are significantly correlated with the severity of severe pneumonia with sepsis, and these levels can be used as important indicators to judge the severity and prognosis of patients.

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