Original Article

Prognostic value of CD4⁺CD25⁺ Tregs as a valuable biomarker for patients with sepsis in ICU

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BACKGROUND: Sepsis is a common complication of infections, burns, traumas, surgeries, poisonings, and post-cardiopulmonary resuscitation. The present study aimed to investigate prognostic value of CD4⁺CD25⁺ regulatory T cells (Treg) in peripheral blood of patients with sepsis.

METHODS: Periphery blood from 28 patients diagnosed with sepsis was collected on day 1 and 7 after hospitalization in the ICU of Shanghai Changzheng Hospital between December 2013 to April 2014. The blood was used for analyses of Treg ratio using flow cytometry and for analyses of blood routine test, C-reactive protein (CRP), bilirubin, procalcitonin (PCT), and coagulation. APACHE II and sequential organ failure assessment (SOFA) scores were also investigated. The results were compared between two outcome groups of survival or death to evaluate prognostic value for sepsis.

RESULTS: The patients had an average age of 60.36±15.03 years, APACHE II score 16.68±7.00, and SOFA score 7.18±3.78. Among the 28 patients, 12 had severe trauma (42.9%), 10 had septic shock (35.7%), and 9 (32.2%) died. The median ratio of Tregs was 2.10% (0.80%, 3.10%) in the survival group vs. 1.80% (1.15%, 3.65%) in the death group ($Z=-0.148$, $P=0.883$) on day 1; however it was significantly changed to 0.90% (0.30%, 2.80%) vs. 5.70% (2.60%, 8.30%) ($Z=-2.905$, $P=0.004$).

CONCLUSION: With better prospects for clinical application, dynamic monitoring of Tregs ratio in peripheral blood has potential value in predicting prognosis of sepsis.

KEY WORDS: CD4⁺CD25⁺ regulatory T cells; Sepsis; Prognosis; Biomarker; APACHE; SOFA; Procalcitonin

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INTRODUCTION

Sepsis is a common complication of infections, burns, traumas, surgeries, poisonings, and post-cardiopulmonary resuscitation. The incidence rate of sepsis is increasing by 1.5%–8.0% annually and its mortality is as high as 30%–70%.^[1,2]

The clinical outcome of sepsis due to infection-induced systemic excessive inflammatory response is largely dependent on the status of host immune function.^[3] During disease progress, sepsis is often accompanied with immune disorders due to dysfunction of T cell subsets including regulatory T cells (Tregs). Tregs as a mature T cell subset play an important role in adaptive immunity to pathogens and autoantigens.^[4,5] Activation of Tregs,

especially CD4⁺CD25⁺ Tregs via TCR stimulation can suppress the body's excessive inflammatory response through inhibition of activation and proliferation of effect T cells, and of antibody production by B cells.^[6,7]

Many studies on CD4⁺CD25⁺ Tregs have focused on signal transductions, but few on its application in clinical use. In this study, we investigated the clinical application of CD4⁺CD25⁺ Tregs in peripheral blood as a potential biomarker in evaluating prognosis of patients with sepsis.

METHODS

Experimental design

Twenty-eight patients with sepsis were observed in

Shanghai Changzheng Hospital from December 2013 to April 2014. The severity of the patients was evaluated according to the acute physiology and chronic health evaluation II (APACHE II) and sequential organ failure assessment (SOFA) scores. Blood samples from the patients were analyzed by flow cytometry for CD4⁺CD25⁺ Tregs. Other related indicators were monitored daily and the worst value in a day was used for analysis.

Criteria of patient selection

Based on 2001 Critically Ill Society, European Society of Critical Care, American College of Chest Physicians, American Thoracic Society, and the Surgical Infection Society International Conference on the definition of systemic infection developed standards (ACCP, SCCM2001), we set up the inclusion criteria. Patients were included if two or more the following items met: 1) body temperature higher than 38 °C or lower than 36 °C; 2) heart rate greater than 90 beats/minute; 3) respiratory rate greater than 30 beats/minute, or PaCO₂ less than 32 mmHg; 4) WBC in peripheral blood greater than 12×10⁹/L or less than 4.0×10⁹/L, or immature granulocytes more than 10%.

The exclusion criteria for patients included: age younger than 18 years; epilepsy; any autoimmune diseases; long-term use of immunosuppressive agents; active bleeding; acute left ventricular failure caused by pulmonary edema.

Collection of blood sample

Blood samples were collected on day 1 and day 7 after hospitalization and treated with heparin. Lymphocytes were isolated and analyzed by flow cytometry for CD4⁺CD25⁺ Tregs ratio. Blood was tested for liver and kidney function, blood clotting, electrolytes, PCT, and endotoxins. If repeated tests occurred in the same day, then the worst value was subjected to further analysis. An etiological test was also conducted on the first day in the ICU.

Analysis of ratio of Tregs in peripheral blood by flow cytometry

All blood samples were analyzed within 30 minutes after collection by flow cytometry according to the instructions of FACS Canto (BD Biosciences, USA) detection and BD FACSDiva software.

Statistical analysis

Normal distribution of measurement data uses mean ±SD and independent sample *t* test was used to compare

the survival group and death group.

Non-normal distribution of measurement data was expressed by the median [M (25th, 75th)], and the Mann-Whitney *U* test and Wilcoxon's nonparametric test were performed between the two groups.

A *P* value<0.05 was considered statistically significant. SPSS19.0 software (SPSS Corp, Chicago, IL, USA) was used for data analysis.

RESULTS

Patient information

In the 28 patients aged 60.36±15.03 years, the average time in ICU was 23.50 (12.25 to 31.75) days. Their APACHE II and SOFA scores on day 1 were 16.68±7.00 and 7.18±3.78 respectively. In this series, 12 (42.9%) patients had severe trauma, 10 (35.7%) had septic shock, and 9 patients (32.2%) died. The major sites of infection of the patients were the lungs (17 patients), bloodstream (10), abdomen (4), urinary tract (3), skin and soft tissue (2), and others (3). Pathogens including gram-negative bacteria and fungi in specimens of body fluids were observed in 22 and 10 patients respectively (Table 1).

Comparison of general blood tests between the survival and death groups

Blood coagulation reaction D-dimer and endotoxin values on day 7 were statistically higher, and platelets were significant lower in the death group than in the survival group (all *P*<0.05) (Table 2). Procalcitonin was significantly

Table 1. The characteristics of patients with sepsis (*n*=28)

Parameters	Value
Age (mean±SD, year)	60.36±15.31
Sex (male/female)	22/6
APACHE II score (mean±SD)	16.68±7.00
SOFA score (mean±SD)	7.18±3.78
ICU LOS [median (25 th and 75 th), days]	23.50 (12.25 and 31.75)
Severe trauma (<i>n</i> , %)	12 (42.9)
Septic shock (<i>n</i> , %)	10 (35.7)
Source of infection (<i>n</i> , %)	
Pneumonia	17 (43.59)
Blood culture positive	10 (35.64)
Intra-abdominal	4 (10.26)
Urinary system	3 (7.69)
Skin and soft tissue	2 (5.13)
Other	3 (7.69)
Pathogen (<i>n</i> , %)	
Gram-negative bacteria	22 (64.71)
Fungus	10 (29.41)
Gram-positive bacteria	2 (5.88)

APACHE II: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment.

higher on day 1 and day 7 in the death group ($P<0.05$).

CD4⁺CD25⁺ Tregs in the survival or death group

The ratios of CD4⁺CD25⁺ Tregs in peripheral blood on day 1 between the two groups were similar. However, they were significantly changed on day 7 to 0.90% (0.30%, 2.80%) in the survival group vs. 5.70% (2.60%, 8.30%) in the death group ($P<0.05$). The difference between day 7 and day 1 (Δ _Treg d7–d1) in the two outcome groups was also statistically significant ($P<0.05$) (Table 3).

DISCUSSION

It is important to diagnose and treat sepsis patients as early as possible in order to achieve favorable clinical outcomes.^[8] Therefore, it is pivotal to accurately predict prognosis of sepsis patients at different stages and to help optimize treatment options. There are few well-established scoring tools including APACHE II score, improved early warning score (modified early warning score, MEWS), and SOFA score,^[9] which have been useful in predicting clinical outcomes. However, it is believed that more effort is needed to find predictive biomarkers at earlier stages such as early changes in immune status in sepsis patients, because immune response has been one of the key factors for the pathogenesis of sepsis.^[10] In patients with sepsis, immune status is not always in a positive static status,^[11] but negative regulation mechanism in the progress of sepsis also plays an important role.^[12] As one of the most important peripheral immune cells, CD4⁺CD25⁺ Treg cells are involved in immune responses in patients with sepsis. The main cause of early death of patients with sepsis is early excessive activation of the immune system to release large amounts of cytokines (cytokine storm) that

damage multi-organ systems.^[13,14] Later deaths are due to reduced antibody-dependent cell-mediated cytotoxicity (ADCC), immunosuppression, decreased ability to clear pathogens,^[5] and increased function of CD4⁺CD25⁺ Tregs suppressing activation of effect T cells.^[11,15]

Our study has shown that the median ratio of CD4⁺CD25⁺ Tregs in peripheral blood on day 1 between the survival and death groups are comparable with 2.10% (0.80%, 3.10%) vs. 1.80% (1.15%, 3.65%). After a week of treatment in the ICU, the ratio was 0.90% (0.30%, 2.80%) vs. 5.70% (2.60%, 8.30%) ($Z=-2.905$, $P=0.004$), with a significantly higher value in the death group than in the survival group. This was supported by the evidence that increased CD4⁺CD25⁺ Tregs may contribute to the worse outcome by suppressing activation of effect T cells even during the treatment. The difference between the two groups [Δ _Tregs (d7–d1)] was also significant, with -0.40% (-1.70% , -0.10%) in the survival group vs. 1.90% (1.55%, 5.10%) in the death group ($Z=-3.351$, $P=0.001$). The reason for this phenomenon may be due to various factors such as traumas, infections, and differences in immune activation in patients. The difference in Δ _Tregs (d7–d1) reflected changes in immune function of patients in a week of disease progress and treatment effects. CD4⁺CD25⁺ Tregs median ratio on day 7 and Δ _Tregs (d7–d1) have indicated their prognostic values as valuable biomarkers in sepsis patients.

In addition, the present study also found that median procalcitonin (PCT) was significantly different between the survival and death groups, with 1.84 ng/mL (0.39 ng/mL, 6.28 ng/mL) in the survival group vs. 8.47 ng/mL (5.59 ng/mL, 14.15 ng/mL) ($Z=-2.730$, $P=0.006$) on day 1 and 0.74 ng/mL (0.21 ng/mL, 2.00 ng/mL) vs. 8.10 ng/mL (4.42 ng/mL, 9.27 ng/mL) ($Z=-3.424$, $P=0.001$) on day 7. The concept of PCT clearance for assessing the prognosis of sepsis introduces the dynamic change of

Table 2. Comparison of platelet, PCT, endotoxin, D-dimer between the survival and death groups

Variables	All patients	Survivors	Non-survivors	Z value	P value
Platelet [median (25 th , 75 th), $\times 10^9/L$]					
Day 1	169.50 (74.50, 228.50)	179.00 (119.00, 250.00)	98.00 (53.50, 192.50)	-1.870	0.062
Day 7	177.00 (127.50, 287.75)	201.00 (163.00, 310.00)	121.00 (60.00, 183.00)	-2.460	0.014
Procalcitonin median [(25 th , 75 th), ng/mL]					
Day 1	4.27 (0.51, 8.26)	1.84 (0.39, 6.28)	8.47 (5.59, 14.15)	-2.730	0.006
Day 7	1.37 (0.26, 5.89)	0.74 (0.21, 2.00)	8.10 (4.42, 9.27)	-3.424	0.001
Endotoxin [day 7, median (25 th , 75 th), EU/mL]	0.68 (0.19, 0.42)	0.12 (0.04, 0.23)	0.48 (0.24, 0.74)	-2.583	0.010
D-dimer [day 7, median (25 th , 75 th), $\mu g/L$]	2705.00 (1012.50, 4580.00)	1930.00 (800.00, 2800.00)	3870.00 (2825.00, 5310.00)	-2.533	0.011

Table 3. Comparison of CD4⁺CD25⁺ Tregs ratio between the survival and death groups

Variables	All patients	Survivors	Non-survivors	Z value	P value
CD4 ⁺ CD25 ⁺ Tregs [day 1, median (25 th , 75 th), %]	2.10 (0.88, 3.10)	2.10 (0.80, 3.10)	1.80 (1.15, 3.65)	-0.148	0.883
CD4 ⁺ CD25 ⁺ Tregs [day 7, median (25 th , 75 th), %]	2.15 (0.70, 4.32)	0.90 (0.30, 2.80)	5.70 (2.60, 8.30)	-2.905	0.004
Δ _Tregs [(day 7–day 1), median (25 th , 75 th), %]	-0.10 (-1.38, 1.85)	-0.40 (-1.70, -0.10)	1.90 (1.55, 5.10)	-3.351	0.001

PCT that is more meaningful than the absolute value of PCT.^[14,16,17] Our study has shown similar results.

Analysis of endotoxins showed that median values on day 7 are also significantly different between the two groups as 0.1240 EU/mL (0.0443 EU/mL, 0.2320 EU/mL) vs. 0.4813 EU/mL (0.2387 EU/mL, 0.7412 EU/mL) ($Z=-2.583$, $P=0.010$). The results are consistent with the published literature.^[18] Reaction of D-dimer coagulation and platelets between the two groups showed a significant difference on day 7, but not day 1, suggesting supplemental values of these indicators for prognosis in sepsis.^[19–21] These changes in PCT, endotoxins, D-dimer coagulation and platelets are useful indicators in monitoring progress of sepsis and provide guidance for timely treatment.

Our study incorporated a small number of cases that are admitted to the ICU but not to general wards in a single study center, therefore sepsis patients in this study had APACHE II scores and SOFA scores that were relatively higher. However, further studies with a larger sample size are needed.

In conclusion, The changes in ratio of CD4⁺CD25⁺ Treg cells in peripheral blood of patients with sepsis are associated with clinical outcomes such as survival or death. CD4⁺CD25⁺ Treg cells may offer a possible biomarker to be able to accurately assess prognosis of sepsis patients.

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