Dynamic Changes in Serum Cortisol and Acth Levels and Lymphocyte Subset Counts in Children With Septic Shock

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Abstract

Backgroud: To study the dynamic changes in serum cortisol, adrenocorticotropic hormone (ACTH) levels and peripheral blood lymphocyte subset counts in children with septic shock (SS) and to explore their association with the severity and prognosis of the disease.

Methods: The levels of serum cortisol, ACTH and peripheral blood lymphocyte subsets were analyzed in 25 patients in the advanced-stage group with decreased blood pressure and 24 patients in the early-stage group with normal blood pressure. Twenty-five healthy children who underwent physical examination were selected as the control group. Children in the advanced-stage group were further divided into the death subgroup (n = 5) and the survival subgroup (n = 20).

Results: At admission, the levels of serum cortisol and ACTH in the advanced-stage group were apparently higher than those in the early-stage group ($P < 0.05$). The advanced-stage group had significantly lower lymphocyte subset counts than the early-stage group ($P < 0.05$). On the 3rd day after admission, the levels of serum cortisol and ACTH in both groups decreased, and the counts of total lymphocytes, T cells and Th cells in the early-stage group were significantly higher than those at admission. On the 8th day after admission, there was no statistically significant difference in the levels of serum cortisol or ACTH between the groups. At admission, there was no statistically significant difference in serum cortisol and ACTH levels between the death subgroup and the survival subgroup. On the third day after admission, the levels of cortisol and ACTH were decreased in both groups, but the differences between the two subgroups were not statistically significant. All the lymphocyte subset counts in the death subgroup on the third day after admission were significantly lower than those in the survival subgroup ($P < 0.05$).

Conclusions: The hypothalamic-pituitary-adrenal axis is excessively activated in children with SS. Higher serum cortisol and ACTH levels and lower peripheral blood lymphocyte subset counts indicate increased severity of the disease. After treatment, the first signs that indicate the effective control of the disease are decreased serum cortisol and ACTH levels and increased T cell, Th cell and total lymphocyte counts.

Background

Septic shock (SS) is one of the most common causes of death in critically ill children. Although great progress has been made in the diagnosis and treatment of septic shock in recent years, its morbidity and mortality rates remain high[1, 2]. The activated immune cells of children with SS produce a large number of inflammatory cytokines, which activate the hypothalamic-pituitary-adrenal (HPA) axis. Immunological cells, which can also synthesize and secrete adrenocorticotropic hormone (ACTH) with immunological competence[3] and cause intensive secretion of cortisol to limit the inflammatory response to protect the body from inflammation and excessive immune response, are crucial safeguards for the body in fighting diseases. In recent years, many studies on the function of the HPA axis at the early stage in pediatric patients with sepsis or severe sepsis have confirmed the clinical significance of serum cortisol and ACTH levels in evaluating the severity of the disease and the prognosis of children with sepsis[4, 5]. The extent of peripheral blood lymphocyte loss in adult patients with sepsis is associated with the severity of the disease and poor prognosis[6]. However, whether these findings are applicable to children with SS should be further studied. In addition, immunosuppression, including cellular and humoral immunity, occurs at a relatively early stage in children with severe sepsis and appears to be due to the apoptosis of a large number of lymphocytes, decreased proliferation of lymphocytes and nonspecific immune dysfunction. These factors may be connected with the severity and rapid progress of the disease. Therefore, we dynamically monitored the levels of serum cortisol and

ACTH and the counts of peripheral blood lymphocyte subsets in children with SS and compared them with those of healthy children to study the connection between them and the severity of the disease to provide sensitive indicators for judgment on clinical efficacy and prognosis prediction.

In daily work, pediatricians in the pediatric intensive care unit (PICU) often make a diagnosis and administer treatment in line with relevant clinical guidelines for adults. Although the guidelines are indeed helpful, they are based on clinical tests on adult patients. Therefore, when applied to children, they must be adjusted according to children's physiological and pathological characteristics. For example, in Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock (2012 edition)[7], the diagnostic criterion for septic shock in children, continuous hypotension induced by sepsis, which is ineffective for fluid resuscitation, is the same as that in adults. However, the hemodynamic characteristics of children are not the same as those of adults. With their resting heart rate of only 70 beats/min, adults have much more powerful cardiac reserve than children. When suffering from heavy blows such as sepsis, the heart rate of adults can double to 140 times/min to increase cardiac output. Hence, almost 90% of adult patients with septic shock appear to have hyperdynamic shock syndrome or warm shock[8], which is characterized by increased heart rate, low systemic vascular resistance (SVR), hypotension, and normal or increased cardiac output. However, the resting heart rate of children is faster than that of adults; therefore, their cardiac reserve is limited. The main response of children to the decrease in cardiac output caused by sepsis is vasoconstriction, which is why 50% of children with septic shock have cold shock, characterized by decreased cardiac output and increased circulatory resistance[9]. Therefore, hypotension tends not to occur in children with septic shock at the early stage[10]. In view of this, in this study, children with septic shock were divided into the early-stage group with normal blood pressure and the advanced-stage group with reduced blood pressure according to the Diagnosis and Treatment Recommendation for the Pediatric Septic Shock in China in 2006.

The following signs indicate tissue hypoperfusion:

(1) Changes in heart rate and pulse: weak peripheral artery and accelerating heart rate and pulse

(2) Changes in skin: pale or pale gray, wet and cold, and marbled complexion, if warm shock: warm limbs and dry skin

(3) Prolonged (> 3 s) capillary refilling time (CRT) (except for the influence of ambient temperature) or normal CRT (if warm shock)

(4) Change in consciousness: fidgety or drooping at the early stage with indifferent expression; ambiguous consciousness at the advanced stage, even coma or convulsion

(5) After fluid resuscitation, urine volume is still less than 0.5 ml/kg/h, lasting for at least two hours.

(6) Lactic acidosis (except for other ischemic, hypoxic and metabolic factors), with lactic acid in arterial blood greater than 2 mmol/L.

After the diagnosis of sepsis in children, the early stage of septic shock is diagnosed if the clinical manifestations meet 3 of the above 6 criteria. The advanced stage of septic shock is diagnosed if there is a blood pressure drop. In 2015, Chinese experts on the diagnosis and treatment of septic shock in children recognized the diagnostic criteria as well by consensus. Recent studies have also verified that blood pressure tends to be normal during the early stage of septic shock in children[11]. To study the dynamic change in serum cortisol and ACTH levels and peripheral blood lymphocyte subsets in children with septic shock (SS), the levels of serum cortisol and ACTH and peripheral

blood lymphocyte subsets were analyzed in the advanced-stage group with decreased blood pressure and in the early-stage group with normal blood pressure on the 1st, 3rd and 8th days after admission to explore their association with the severity and prognosis of the disease.

Methods

Instruments and Reagents

Cortisol was detected with an ACCESS-type automatic chemiluminescence immune detector, which was produced by Beckman (U.S.). ACTH was detected with a LIASION-type automatic chemiluminescence immune detector, which was produced by DiaSorin (Italy). We used only the original kits. Lymphocyte subsets were determined with the BO ll-type flow cytometer manufactured by BD Biosciences (U.S.). Both the antibodies and hemolysin were also products of BD Biosciences.

Study population and design

This was a retrospective study. The data for this study were collected from patients with SS admitted to the PICU of Fujian Maternal and Child Health Care Hospital (FMCHCH), the largest specialized hospital for children in Fujian Province, excluding children with immunodeficiency disease or tumors and children who had recently received immunosuppressive therapy. Healthy children of the similar age who came in for physical examinations at the same period were selected as the control group. After admission, the SS patients in both the advanced-stage group and the early-stage group underwent fluid resuscitation, respiratory support, anti-infection treatment, organ protection and symptomatic support therapies, vasoactive drug therapy, and low-dose glucocorticoid therapy. Ethics board approval was obtained from the Hospital Ethics Committee of FMCHCH, Affiliated Hospital of Fujian Medical University (2014-101). The need for informed consent was exempted because of the retrospective nature of the study.

Detection metrics and methods

Fasting venous blood was drawn on the day of admission and between 8:00 a.m. and 9:00 a.m. on the third and eighth days after admission from the children in both the advanced-stage group and the early-stage group. Fasting venous blood was drawn from the children in the control group between 8:00 a.m. and 9:00 a.m. on the day of physical examination. Two milliliters of blood was collected into an coagulant tube and an EDTA-2K anticoagulant tube and centrifuged for 10 minutes to separate the blood serum and plasma. The blood serum and plasma samples were then transferred to 1.5 ml centrifuge tube and stored at -70°C to determine blood cortisol and ACTH by chemiluminescent method following the instructions for the kits. Another 1.5 ml of venous blood was drawn and collected into an EDTA anticoagulant tube for determinations of lymphocyte subsets with a flow cytometer.

Statistical Analysis

The variables with normal distribution are presented as the mean ± the standard deviation, and the variables that deviated from normal distribution are presented as the medians and 25^{th} and 75^{th} percentiles.

Student's *t*-tests and one-way analysis of variance (ANOVA) were applied to compare the baseline data between two SS groups or among all three groups (the two SS groups plus the control group) when the data were normally distributed. For data that deviated from a normal distribution, Mann-Whitney *U* tests and Kruskal-Wallis rank-sum

tests were used for baseline data comparisons between two or among three groups. Fisher's exact tests were applied for categorical data comparisons.

After the logarithmic transformation of the metrics that deviated from a normal distribution, repeated-measure ANOVA was adopted to evaluate the variation between the two SS groups. To detect the differences in blood metrics among all three groups on the 1st day of hospitalization, the P-values for multiple comparisons were calculated using the Bonferroni method.

Mann-Whitney U tests were used to compare the medians of metrics between the two SS groups on the 1st, 3rd and 8th days. All P-values for multiple comparisons were corrected by the Bonferroni method.

All statistical tests were two-sided with a significance level of 0.05. SPSS 19.0 software was adopted for data processing and analysis.

Results

From December 2014 to December 2016, a total of 49 children with SS were admitted to the PICU of our hospital, including 27 males and 22 females. According to the recommended protocol for the diagnosis and treatment of septic shock in children in China in 2006, these children were divided into the advanced-stage group with decreased blood pressure (25 patients, including 14 males and 11 females) and the early-stage group with normal blood pressure (24 patients, including 13 males and 11 females). In addition, 25 healthy children (including 13 males and 12 females) of the corresponding age were randomly selected as the control group. There were no statistically significant differences in age (P = 1.000), sex (P = 0.962), weight (P = 0.326), *etc*. (Table 1). There was no significant difference in infection sites between the advanced-stage group and the early-stage group (P = 1.000, Table 1). There were 5 deaths in the advanced-stage group and no deaths in the early-stage group. There were significant differences in pediatric critical illness scores, shock correction time, and cortisol and ACTH levels at admission between the two groups (P < 0.001, Table 1). At admission, the lymphocyte count in the early-stage group was less than that in the control group, while that in the advanced-stage group was less than that in the early-stage group (P < 0.001, Table 1).

Table 1
Patient demographics

Variables	Normal	Early Stage	Advanced stage	*P*-value §	*P*-value §§
Sex *				1.000	1.000
Male	14 (56.0%)	13 (54.2%)	14 (56.0%)		
Female	11 (44.0%)	11 (45.8%)	11 (44.0%)		
Age (month) #	19.2 ± 6.6	19.7 ± 7.2	19.5 ± 7.3	0.928	0.962
Weight (kg) ##	11.0 (10.0, 12.0)	12.0 (11.0, 12.7)	12.0 (11.0, 12.0)	0.539	0.326
Infection site *				1.000	—
Lung	—	13 (54.2%)	13 (52.0%)		
Intestinal	—	5 (20.8%)	5 (20.0%)		
Intracranial	—	5 (20.8%)	6 (24.0%)		
Urinary	—	1 (4.2%)	1 (4.0%)		
Hypotension *				< 0.001	—
Yes	—	0 (0.0%)	25 (100%)		
No	—	24 (100%)	0 (0.0%)		
Death *				0.050	—
Yes	—	0 (0.0%)	5 (20.0%)		
No	—	24 (100%)	20 (80.0%)		
PCIS ##	—	74 (73, 76)	71 (67, 72)	< 0.001	—
SCT (hour) ##	—	3.1 (2.7, 3.4)	3.7 (3.4, 4.6)	< 0.001	—
Cortisol level (μg/dl) #	—	32.6 ± 5.2	50.7 ± 6.5	< 0.001	—
ACTH (pg/ml) ##	—	23.0 (19.5, 26.0)	72.0 (69.0, 75.0)	< 0.001	—

PCIS, Pediatric Critical Illness Score; SCT, shock correction time.

* Presented as the frequency (percentage); Fisher's exact test was applied.

Presented as the mean ± the standard deviation for the variables with normal distribution; the one-way ANOVA was applied for the comparison of the mean among three groups, and the independent-sample t-tests were applied for comparison of means between the early- and advanced-stage groups.

Presented as the median (25th percentile, 75th percentile) for the variables that deviated from normal distribution; the Kruskal-Wallis rank-sum tests were applied for comparisons among three groups, and the Mann-Whitney U tests were used to test the differences in medians between the early- and advanced-stage groups.

§ P-values for statistical tests between the early-stage and advanced-stage groups, §§ P-values for statistical tests among three groups.

Variables	Normal	Early Stage	Advanced stage	*P*-value §	*P*-value §§
Lymphocyte (10^9/L) ##	6.2 (6.0, 6.2)	3.5 (3.4, 3.8)	2.4 (2.4, 2.5)	< 0.001	< 0.001

PCIS, Pediatric Critical Illness Score; SCT, shock correction time.

* Presented as the frequency (percentage); Fisher's exact test was applied.

Presented as the mean ± the standard deviation for the variables with normal distribution; the one-way ANOVA was applied for the comparison of the mean among three groups, and the independent-sample t-tests were applied for comparison of means between the early- and advanced-stage groups.

Presented as the median (25th percentile, 75th percentile) for the variables that deviated from normal distribution; the Kruskal-Wallis rank-sum tests were applied for comparisons among three groups, and the Mann-Whitney U tests were used to test the differences in medians between the early- and advanced-stage groups.

§ P-values for statistical tests between the early-stage and advanced-stage groups, §§ P-values for statistical tests among three groups.

The dynamic changes in serum cortisol and ACTH levels in the advanced- and early-stage groups and their comparison with those in the control group

AAt admission, there were statistically significant differences in serum cortisol levels between the advanced-stage group and the early-stage group (P < 0.001, Table 2). The cortisol levels in the advanced-stage group were higher than those in the early-stage group on the first day and the third day (Table 3). The cortisol levels decreased with the time after admission (Fig. 1A), and there was no difference between the two groups on the eighth day (P = 1.000, Table 3).

Table 2
Dynamic Changes in Serum Cortisol and ACTH Levels and Lymphocyte Subset Counts

Variables	Groups	Time			*P*-Values §			Multiple Comparison *P*-Values †	
		1st day	3rd day	8th day	BG	BT	INT	Control	Early SS
Cortisol level (μg/dl) #					< 0.001	< 0.001	< 0.001		
	Early Stage	32.58 ± 5.25	15.54 ± 3.64	14.21 ± 2.25					
	Advanced Stage	50.72 ± 6.53	34.88 ± 5.49	15.30 ± 2.66					
ACTH (pg/ml) ##					< 0.001	< 0.001	< 0.001		
	Early Stage	23.0 (19.5, 26.0)	20.5 (17.5, 22.5)	13.5 (11.0, 15.0)					
	Advanced Stage	72.0 (69.0, 75.0)	32.0 (29.0, 35.0)	15.0 (13.0, 16.0)					
Lymphocyte (10^9/L) ##					< 0.001	< 0.001	0.347		
	Control	6.17 (5.99, 6.24)							
	Early Stage	3.54 (3.43, 3.76)	4.55 (4.11, 4.71)	5.72 (5.59, 5.97)				< 0.001	
	Advanced Stage	2.44 (2.36, 2.53)	1.86 (1.79, 2.10)	3.95 (3.72, 4.20)				< 0.001	< 0.001
B Cell (10^9/L) #					0.951	< 0.001	< 0.001		

Presented as the mean ± the standard deviation for the variables with a normal distribution.

Presented as the median (25th percentile, 75th percentile) for the variables that deviated from a normal distribution.

§ Repeated-measures ANOVA was used to analyze the mean differences between groups (BG) and between time (BT) and the interactions between group and time (INT). The control group was excluded from this analysis because the items listed in the above table were checked only on the 1st day. For those variables that deviated from a normal distribution, the data were transformed by natural logarithm and followed by repeated-measures ANOVA.

† One-way ANOVA was applied for the mean differences in tests for the blood lymphocyte count (total lymphocyte, B cell, T cell, Th cell, Ts cell, and NK cell counts) among three groups, and then the P-values for multiple comparisons were calculated by using the Bonferroni method. For those variables that deviated from a normal distribution, the data were transformed by natural logarithm and followed by one-way ANOVA.

Variables	Groups	Time			*P*-Values §			Multiple Comparison *P*-Values †	
		1st day	3rd day	8th day	BG	BT	INT	Control	Early SS
	Control	1.43 ± 0.08							
	Early Stage	0.91 ± 0.14	1.10 ± 0.11	1.26 ± 0.04				< 0.001	
	Advanced Stage	1.12 ± 0.11	0.90 ± 0.14	1.16 ± 0.05				< 0.001	< 0.001
T Cell (10^9/L) ##					< 0.001	< 0.001	< 0.001		
	Control	4.12 (3.88, 4.28)							
	Early Stage	2.04 (1.96, 2.08)	3.02 (2.97, 3.05)	3.72 (3.54, 3.88)				< 0.001	
	Advanced Stage	1.12 (1.08, 1.18)	0.78 (0.71, 0.83)	2.55 (2.48, 2.59)				< 0.001	< 0.001
Th Cell (10^9/L) ##					< 0.001	< 0.001	< 0.001		
	Control	2.23 (2.18, 2.26)							
	Early Stage	1.12 (1.06, 1.18)	2.32 (2.20, 2.36)	2.32 (2.24, 2.38)				< 0.001	
	Advanced Stage	0.72 (0.69, 0.77)	0.52 (0.47, 0.58)	2.03 (1.97, 2.14)				< 0.001	< 0.001

Presented as the mean ± the standard deviation for the variables with a normal distribution.

Presented as the median (25th percentile, 75th percentile) for the variables that deviated from a normal distribution.

§ Repeated-measures ANOVA was used to analyze the mean differences between groups (BG) and between time (BT) and the interactions between group and time (INT). The control group was excluded from this analysis because the items listed in the above table were checked only on the 1st day. For those variables that deviated from a normal distribution, the data were transformed by natural logarithm and followed by repeated-measures ANOVA.

† One-way ANOVA was applied for the mean differences in tests for the blood lymphocyte count (total lymphocyte, B cell, T cell, Th cell, Ts cell, and NK cell counts) among three groups, and then the P-values for multiple comparisons were calculated by using the Bonferroni method. For those variables that deviated from a normal distribution, the data were transformed by natural logarithm and followed by one-way ANOVA.

Variables	Groups	Time			*P*-Values §			Multiple Comparison *P*-Values †	
		1st day	3rd day	8th day	BG	BT	INT	Control	Early SS
Ts Cell (10^9/L) ##					< 0.001	< 0.001	< 0.001		
	Control	1.92 (1.88, 1.96)							
	Early Stage	0.75 (0.71, 0.81)	0.72 (0.70, 0.79)	1.36 (1.29, 1.43)				< 0.001	
	Advanced Stage	0.31 (0.29, 0.32)	0.21 (0.19, 0.26)	0.32 (0.30, 0.34)				< 0.001	< 0.001
NK Cell (10^9/L) #					< 0.001	< 0.001	< 0.001		
	Control	0.71 ± 0.12							
	Early Stage	0.70 ± 0.07	0.63 ± 0.08	0.82 ± 0.10				1.000	
	Advanced Stage	0.23 ± 0.05	0.22 ± 0.05	0.22 ± 0.02				< 0.001	< 0.001

Presented as the mean ± the standard deviation for the variables with a normal distribution.

Presented as the median (25th percentile, 75th percentile) for the variables that deviated from a normal distribution.

§ Repeated-measures ANOVA was used to analyze the mean differences between groups (BG) and between time (BT) and the interactions between group and time (INT). The control group was excluded from this analysis because the items listed in the above table were checked only on the 1st day. For those variables that deviated from a normal distribution, the data were transformed by natural logarithm and followed by repeated-measures ANOVA.

† One-way ANOVA was applied for the mean differences in tests for the blood lymphocyte count (total lymphocyte, B cell, T cell, Th cell, Ts cell, and NK cell counts) among three groups, and then the P-values for multiple comparisons were calculated by using the Bonferroni method. For those variables that deviated from a normal distribution, the data were transformed by natural logarithm and followed by one-way ANOVA.

Table 3
The early-stage group vs. the advanced-stage group

Variables	Groups	1st day			3rd day			8th day *		
		M (P_{25}, P_{75})	Z§	*P*#	M (P_{25}, P_{75})	Z	*P*#	M (P_{25}, P_{75})	Z	*P*#
Cortisol	Early SS	33.0 (30.0, 35.5)	5.825	< 0.001	15.0 (12.0, 18.0)	6.005	< 0.001	13.5 (12.0, 16.0)	1.346	1.000
	Late SS	52.0 (47.0, 55.0)			35.0 (31.0, 39.0)			15.0 (13.5, 17.5)		
ACTH	Early SS	23.0 (19.5, 26.0)	6.006	< 0.001	20.5 (17.5, 22.5)	5.958	< 0.001	13.5 (11.0, 15.0)	1.035	1.000
	Late SS	72.0 (69.0, 75.0)			32.0 (29.0, 35.0)			15.0 (13.0, 16.0)		
Lymphocyte	Early SS	3.54 (3.43, 3.76)	5.801	< 0.001	4.55 (4.11, 4.71)	6.001	< 0.001	5.72 (5.59, 5.97)	5.468	< 0.001
	Late SS	2.44 (2.36, 2.53)			1.86 (1.79, 2.10)			3.95 (3.72, 4.20)		
B cell	Early SS	0.82 (0.87, 0.96)	4.762	< 0.001	1.11 (1.04, 1.18)	4.452	< 0.001	1.26 (1.23, 1.29)	4.932	< 0.001
	Late SS	1.12 (1.06, 1.18)			0.91 (0.86, 0.93)			1.17 (1.13, 1.19)		
T cell	Early SS	2.04 (1.96, 2.08)	6.001	< 0.001	3.02 (2.97, 3.05)	6.004	< 0.001	3.72 (3.54, 3.88)	5.658	< 0.001
	Late SS	1.12 (1.08, 1.18)			0.78 (0.71, 0.83)			2.55 (2.48, 2.59)		
Th cell	Early SS	1.12 (1.06, 1.18)	5.923	< 0.001	2.32 (2.20, 2.36)	6.002	< 0.001	2.32 (2.24, 2.38)	5.176	< 0.001

* Five patients in the advanced-stage group died before the 8th day; therefore, only the 20 surviving patients were used for the following statistical analysis.

§ Two independent-sample Mann-Whitney rank-sum tests were applied to test the differences in medians between the two groups.

All *P*-values were corrected by Bonferroni correction, and the corrected *P* = original *P* × 24 (the number of comparisons).

	Late SS	0.72 (0.69, 0.77)			0.52 (0.47, 0.58)			2.03 (1.97, 2.14)		
Ts cell	Early SS	0.75 (0.71, 0.81)	6.008	< 0.001	0.72 (0.70, 0.49)	6.009	< 0.001	1.36 (1.29, 1.43)	5.662	< 0.001
	Late SS	0.31 (0.29, 0.32)			0.21 (0.19, 0.26)			0.32 (0.30, 0.34)		
NK cell	Early SS	0.71 (0.64, 0.76)	6.006	< 0.001	0.62 (0.57, 0.72)	6.006	< 0.001	0.86 (0.72, 0.91)	5.663	< 0.001
	Late SS	0.22 (0.20, 0.27)			0.22 (0.19, 0.26)			0.23 (0.21, 0.24)		

* Five patients in the advanced-stage group died before the 8th day; therefore, only the 20 surviving patients were used for the following statistical analysis.

§ Two independent-sample Mann-Whitney rank-sum tests were applied to test the differences in medians between the two groups.

All *P*-values were corrected by Bonferroni correction, and the corrected P = original $P \times 24$ (the number of comparisons).

At admission, the ACTH levels in the advanced-stage group were higher than those in the early-stage group ($P < 0.001$, Table 2). On the third day after admission, the ACTH levels in both the advanced-stage group and the early-stage group decreased, but the difference between the two groups was still statistically significant ($P < 0.001$, Table 3) and those in the advanced-stage group were still higher than those in the early-stage group (Fig. 1B). There was no statistically significant difference between the two groups on the eighth day after admission ($P = 1.000$, Table 3).

The dynamic changes in peripheral blood lymphocyte subset counts in the advanced- and early-stage group and their comparison with those in the control group

At admission, the counts of T cells, Th cells, Ts cells and NK cells in the advanced- and early-stage groups and the control group showed significant differences between groups, between time and in the interactions between groups and time ($P < 0.001$, Table 2). The differences between groups and between time for total lymphocyte counts in the three groups were significant ($P < 0.001$, Table 2), but the interactions between group and time were not significant ($P = 0.347$, Table 2). There was no significant difference between groups in B cell counts ($P = 0.951$, Table 2), while there were significant differences between time and the interactions between group and time ($P < 0.001$, Table 2).

At admission, the counts of total lymphocytes, T cells, Th cells, Ts cells and B cells in the advanced- and early-stage groups were significantly lower than those in the control group, and the differences among groups were statistically significant ($P < 0.001$, Table 2). All the counts in the advanced-stage group were significantly lower than those in the early-stage group ($P < 0.001$, Table 3). The NK cell counts in the early-stage group were not significantly different from those in the control group ($P = 1.000$, Table 2), while those in the advanced-stage group were significantly lower than those in the control group ($P < 0.001$, Table 2).

On the third day after admission, the counts of total lymphocytes, T cells and Th cells in the early-stage group were significantly higher than those at admission (Fig. 1C, E and F). Except for NK cells, the counts of other lymphocyte subsets (total lymphocyte, T cell, Th cell, Ts cell and B cell counts) in the advanced-stage group were significantly lower than those at admission (Fig. 1C, D, E, F and G). Among them, all the counts in the advanced-stage group were significantly lower than those in the early-stage group ($P < 0.001$, Table 3).

On the eighth day after admission, the total lymphocyte, Th cell and NK cell counts in the early-stage group showed no statistical significance compared with the control group (Fig. 1C, F, H). Other subgroups showed a significant increase, but the differences with the control group were still statistically significant (Fig. 1D, E, G). The counts of Th cells, T cells and total lymphocytes in the advanced-stage group were significantly higher than those at admission ($P < 0.001$, Table 2). The counts of Ts cells, B cells and NK cells increased, but there was no statistical significance ($P > 0.05$, Table 2).

Except for B cell counts at admission and on the eighth day, all lymphocyte subset counts at any time point in the advanced-stage group were smaller than those in the early-stage group ($P < 0.001$, Table 3).

Comparison of the dynamic changes of serum cortisol and ACTH in the death and survival subgroups

Five patients in the advanced-stage group died on the third to fifth day after admission and were classified into the death subgroup, and the remaining 20 patients were classified into the survival subgroup. At admission, there was no statistically significant difference in serum cortisol levels ($P = 0.226$) or ACTH levels ($P = 0.426$) between the two groups (Table 4). On the third day after admission, the levels of cortisol and ACTH were decreased in both groups, but the differences between the two groups were not statistically significant (Table 4).

Table 4
The death subgroup vs. the survival subgroup

Variables	Cases	1st day			3rd day		
		M (P_{25}, P_{75})	Z§	*P*#	M (P_{25}, P_{75})	Z§	*P*#
Cortisol	Survived	49.0 (45.5, 53.0)	2.454	0.226	33.0 (30.5, 36.0)	2.927	0.055
	Died	57.0 (53.0, 60.0)			41.0 (39.0, 44.0)		
ACTH	Survived	71.0 (66.0, 74.0)	2.217	0.426	32.0 (28.5, 34.5)	2.147	0.509
	Died	75.0 (74.0, 79.0)			35.0 (34.0, 37.0)		
Lymphocyte	Survived	2.46 (2.41, 2.63)	3.264	0.018	1.94 (1.83, 2.40)	3.330	0.014
	Died	2.19 (2.19, 2.22)			1.08 (1.02, 1.13)		
B cell	Survived	1.15 (1.11, 1.19)	3.263	0.018	0.92 (0.89, 0.98)	3.403	0.011
	Died	1.01 (0.95, 1.02)			0.72 (0.70, 0.72)		
T cell	Survived	1.14 (1.10, 1.19)	2.652	0.128	0.79 (0.77, 0.84)	3.403	0.011
	Died	0.92 (0.89, 1.09)			0.54 (0.53, 0.60)		
Th cell	Survived	0.73 (0.71, 0.78)	2.281	0.361	0.53 (0.51, 0.59)	3.369	0.012
	Died	0.62 (0.57, 0.71)			0.39 (0.36, 0.40)		
Ts cell	Survived	0.32 (0.30, 0.34)	2.191	0.455	0.23 (0.21, 0.28)	3.186	0.023
	Died	0.28 (0.27, 0.30)			0.16 (0.15, 0.18)		
NK cell	Survived	0.24 (0.21, 0.28)	3.040	0.038	0.23 (0.21, 0.28)	3.311	0.015
	Died	0.18 (0.16, 0.19)			0.16 (0.12, 0.17)		

§ Two independent-sample Mann-Whitney rank-sum tests were applied to test the differences in medians between the survival subgroup and the death subgroup in the advanced-stage group.

All *P*-values were corrected by Bonferroni correction, and the corrected *P* = original *P* × 16 (the number of comparisons).

Comparison of the dynamic changes in the peripheral blood lymphocyte subset counts in the death and survival groups

At admission, the counts of total lymphocytes, B cells and NK cells in the death subgroup were lower than those in the survival subgroup (all $P < 0.05$, Table 4), while there was no statistically significant difference between the two subgroups in the other lymphocyte subgroup counts (T cells, Th cells and Ts cells) ($P > 0.05$, Table 4). All the counts of lymphocyte subsets in the death group on the third day after admission were significantly lower than those in the survival group ($P < 0.05$, Table 4).

Discussion

The neuroendocrine system and immune system influence and regulate each other under stress. Research suggests that when sepsis occurs, the HPA axis can be stimulated by the body's neuroendocrine regulation mechanism,

promoting the pituitary to release ACTH to act on the adrenal cortex. Through short feedback adjustment, ACTH can self-regulate its release. An increase in ACTH levels is the body's first response to damage to adrenal cortex function[12] and is a compensatory expression of the body's efforts to maintain normal adrenal cortical hormone levels. Cortical hormone is produced and released to limit the inflammatory response[13], inhibiting the cytokine cascade reaction produced by the excessive activation of lymphocytes and preventing circulatory failure and multiple organ dysfunction syndrome. Cortical hormone plays a negative feedback role in regulating the secretion of corticotropin releasing hormone (CRH) in the hypothalamus and ACTH in the anterior pituitary gland. The serum cortical hormone level of patients with severe infection generally rises, and the fluctuation range apparently expands[14]. Boonen[15] found that the expression and activity of cortisol metabolic enzymes in sepsis patients decreased, resulting in a decrease in cortisol decomposition in plasma and an increase in cortisol. However, as target organs cannot make full use of the existing adrenal cortical hormone, the body can hardly manage the state of stress; this is known as relative adrenocortical insufficiency (RAI)[16], which appears to be the state of "sufficient hunger".

Sam[17] compared and observed the levels of free cortisol in patients with SS, patients with sepsis and a control group. The results suggested that the levels of free cortisol in SS patients were apparently higher than those in sepsis patients or the control group, indicating that the change in cortisol level in the body was closely associated with the severity of the disease. Because of the difficulty in determining free cortisol levels in blood, the serum total cortisol level is often chosen as the indicator of adrenal cortex function in clinical practice. Our research showed that the serum cortisol levels of the patients in the advanced- and early-stage groups were apparently higher than those in the control group, and they were significantly higher in the advanced-stage group than in the early-stage group. With the time of admission the control of infection, on the third day after admission, serum cortisol levels dropped in both the advanced- and early-stage groups, but they were still significantly higher in the advanced-stage group than in the early-stage group and the control group. There were no statistically significant differences between the early-stage group and the control group in serum cortisol levels. On the eighth day after admission, there were no statistically significant differences among the three groups in serum cortisol levels. The serum cortisol levels in the death subgroup were significantly higher than those in the survival subgroup on admission and the third day after admission. These results suggested that the serum cortisol levels of children with septic shock are positively correlated with the severity of the disease. Those whose serum cortisol level quickly drops to the normal level have a favorable prognosis, and a continuously high level of serum cortisol indicates a high risk of death.

Many studies[4, 5] have found that, on admission, higher levels of ACTH indicate increased severity of disease. Moreover, the level of ACTH decreases in convalescence, indicating that the increasing level of ACTH is associated with the severity of the disease. Through autopsies of patients who died of SS, Sharshar[18] found that excessive inflammatory mediators could induce the apoptosis of pituitary cells, resulting in a sharp reduction in the synthesis and secretion of ACTH. Our research suggested that in both the advanced- and the early-stage groups, the admission levels of ACTH were apparently higher than those in the control group, and they were significantly higher in the advanced-stage group than in the early-stage group. Over time, on the third day after admission, the levels of ACTH dropped in both the advanced- and the early-stage groups with the control of infection. However, they were still apparently higher in the advanced-stage group than in either the early-stage group or the control group. Additionally, they were higher in the early-stage group than in the control group. On the eighth day after admission, there were no statistically significant differences among the three groups in ACTH levels. The admission levels of ACTH in the death subgroup were significantly higher than those in the survival subgroup. On the third day after admission, the ACTH levels in both subgroups dropped, especially in the death subgroup. There was no significant

difference in ACTH levels between the subgroups. The above results suggest that the ACTH levels of children with septic shock are positively correlated with the severity of the disease.

On the other hand, in patients with SS, lymphocytes are excessively activated, resulting in a cytokine cascade reaction, which leads to circulatory failure or even multiple organ dysfunction syndrome. The lymphocyte subset counts (including total lymphocytes, $CD3^+$ T cells, $CD4^+$ Th cells, $CD8^+$ Ts cells, $CD19^+$ B cells and $CD56^+$ NK cells) are negatively correlated with immune cell apoptosis and can be used to monitor whether the immune system is active or suppressed. CD3 molecules are expressed on the surface of all mature T cells, and $CD3^+$ T cells represent all T cells[19]. $CD4^+$ T cells are the hub in the regulation of the body's immune function, and they are the main cells involved in cellular immunity. After being activated, $CD4^+$ T cells can further mature and differentiate into two functional subgroups, namely, Th1 and Th2 cells. The functional subgroups are interconditioned. The former induces the transformation of effector cells to perform the function of cellular immunity, while the latter can express the membrane protein CD40L, which combines CD40 on $CD19^+$ B cells to enhance the activity of B cells. In this way, $CD4^+$ T cells are indirectly involved in B cell-dominated humoral immunity and induce the production of antibodies and the conversion and secretion of allotype. Decreasing $CD4^+$ T cell levels can cause the inhibition of cellular and humoral immunity. $CD8^+$ T cells are divided into inhibitory T cells (Ts) and cytotoxic T cells (Tc). The former inhibit immunity and lead to self-limited changes in lymphocyte proliferation, while the latter directly kill target cells, which appears to be a bidirectional adjustment. Activated B cells are also antigen-presenting cells that can activate T cells and promote their proliferation and activation. $CD56^+$ NK cells are natural killer cells, which are the first response cells and play an important role in immune surveillance and early anti-infection immunity. NK cells are first activated by the mRNA of cytokines and then rapidly induce the production of cytokines. In this way, they have certain inhibitory effects on the proliferation and differentiation of T cells and B cells[20].

Timperi E[21] indicated that the levels of $CD3^+$ T, $CD4^+$ T and $CD8^+$ T cells and the $CD4^+$ T/$CD8^+$ T ratios in children with severe sepsis were apparently lower than those in children with mild sepsis. The levels of $CD3^+$ T, $CD4^+$ T and $CD8^+$ T cells and the $CD4^+$ T/$CD8^+$ T ratios in children in the death group with sepsis were apparently lower than those in the survival group, with the extent of reduction associated with the severity of the disease. Gogos[22] found that the lymphocyte subset counts, including NK and B cells, in severe sepsis patients with community-acquired pneumonia and abdominal infection were apparently lower than those in mild sepsis patients, especially the $CD4^+$ T cell counts. The data of our study showed that the total lymphocyte counts and the $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, $CD19^+$ B and $CD56^+$ NK cell counts in the advanced- and early-stage groups apparently decreased on admission, with the levels in the advanced-stage group being the lowest. The differences among the three groups were statistically significant. This finding indicates that the immunosuppressive state occurs in the early stage of SS in children. Lower absolute values of each lymphocyte subgroup are observed with increasing severity of disease. This finding may be in connection with the following factors: (1) the dissolution of activated and immature T cells (thymus cells and transformed lymphocytes) through apoptosis; (2) the production of a large amount of adrenal cortical hormone by the activation of the hypothalamic-pituitary-adrenal axis produces a large amount of adrenal cortical hormone, resulting in the inhibition of the immune function of the body; and (3) the redistribution of lymphocytes in circulation to other lymphoid tissues.

Hotchkiss[23] reported that the apoptosis of lymphocytes was apparently increased in the lymphatic organs (spleen and lymph nodes) of patients who died of sepsis, and there was obvious decreased lymphocytosis. The apoptotic cells were mainly $CD4^+$ T, B and dendritic cells. Ibrahiem[24] reported that the levels of $CD3^+$ T and $CD4^+$ T cells and

the $CD4^+$ T/$CD8^+$ T ratios in patients who died of severe sepsis were continuously low. In our study, on the third day after admission, the counts of total lymphocytes and $CD3^+$ T, $CD4^+$ T, $CD8^+$ T, $CD19^+$ B, and $CD56^+$ NK cells in the death group significantly decreased, appearing to be extremely low, and were significantly different from those in the survival group. This suggests that the continuous intensification of the immunosuppressive state indicates poor prognosis in children with SS. Additionally, this study found that the immune function of the children in the survival group was gradually restored, and the first sign was the apparent increase in the counts of $CD3^+$ T and $CD4^+$ T cells and total lymphocytes. The statistically significant increase occurred in the early-stage group on the third day after admission and in the advanced-stage group on the eighth day, especially the increase in $CD4^+$ T cells. On the eighth day after admission, the $CD4^+$ T cell count in the early-stage group rose to normal levels.

In summary, higher levels of serum cortisol and ACTH in children with SS indicate increased severity of the disease. Continuously high levels of serum cortisol indicate a poor prognosis and a high risk of death. Furthermore, lower peripheral blood lymphocyte subset counts indicate increased severity of the disease. Continuously low peripheral blood lymphocyte subset counts indicate a high risk of death. After treatment, the rise in the counts of $CD4^+$ T cells, $CD3^+$ T cells and total lymphocytes, especially the increase in the $CD4^+$ T cell count, is the first sign of effective disease control. Therefore, dynamic monitoring of serum cortisol and ACTH levels and lymphocyte subsets, especially of serum cortisol levels and lymphocyte subset counts, in children with SS is of great clinical significance in evaluating the severity and prognosis of the disease.

Conclusions

The hypothalamic-pituitary-adrenal axis is excessively activated in children with SS. Higher serum cortisol and ACTH levels and lower peripheral blood lymphocyte subset counts indicate increased severity of the disease. After treatment, the first signs that indicate the effective control of the disease are decreased serum cortisol and ACTH levels and increased T cell, Th cell and total lymphocyte counts.

Abbreviations

ACTH
adrenocorticotropic hormone; CRT:capillary refilling time; CRH:corticotropin releasing hormone; HPA:hypothalamic-pituitary-adrenal; SS:septic shock; SVR:systemic vascular resistance; RAI:relative adrenocortical insufficiency

Declarations

Ethics approval and consent to participate

This study was approved by the Hospital Ethics Committee of Fujian Provincial Maternity and Children's Hospital, Affiliated Hospital of Fujian Medical University (2014 – 101).The need for informed consent was exempted because of the retrospective nature of the study.

Consent for publication

Not applicable.

Availability of data and material

Anonymous data used in this study is available upon request from the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ML collected data, wrote the draft and final version of the manuscript. BQZ carried out statistical analyses and interpreted data collected data. HL designed the study, supervised the whole process and wrote the final version of the manuscript. PMS critically revised the manuscript. SBW, RMG, BW, and XFG collected data. All authors read and approved the final version of the manuscript.

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Publisher's Note

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Figures

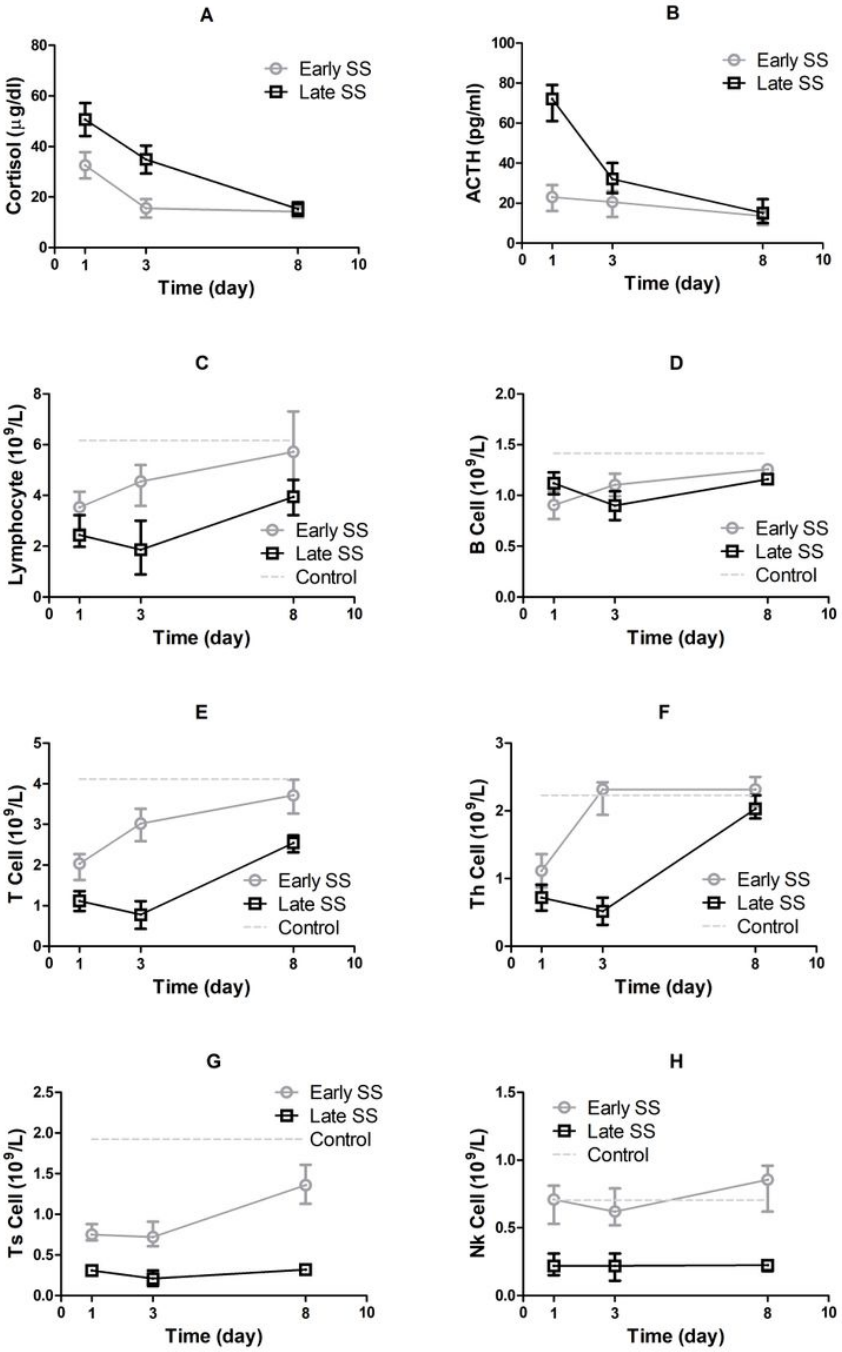

Figure 1

Dynamic changes in the metrics in every group. “Early SS” means the early-stage group, “Late SS” means the advanced-stage group, “Control” means the control group. The horizontal dashed lines in C to H indicate the median values of the metrics from the control group. The boxes and bars in A to H represent the medians and the upper (P75) and lower quartiles (P25). The mean differences in cortisol levels between the early-stage and advanced-stage groups tended to shrink on the 8th day (A), and a similar trend in variation was also detected in ACTH levels (B). The mean differences in lymphocyte counts between the early-stage and advanced-stage groups tended to amplify on the third day and shrink on the 8th day (C), and a similar trend in variation was also detected in B cells, T cells and Th cells (D, E, F). The mean differences in Ts cell and NK cell counts between the early-stage and advanced-stage groups tended to shrink on the third day and to amplify on the 8th day (G, H).