Early Changes in Circulatory T Helper Type 1, 2, and 17 Cells of Patients with Out-of-Hospital Cardiac Arrest after Successful Cardiopulmonary Resuscitation

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Abstract

Background: Immune disorder is an important feature of patients with out-of-hospital cardiac arrest (OHCA) after the return of spontaneous circulation (ROSC). We investigated the expression of circulatory T helper type (Th) 1, Th2, and Th17 cells to explore the early immune alteration in OHCA patients after ROSC.

Methods: During July–September 2016 and March–September 2017, 65 consecutive OHCA patients with ROSC >12 h and 30 healthy individuals were enrolled in this study. Clinical and 28-day survival data were collected. Peripheral blood samples were analyzed to evaluate the expression of Th1/Th2/Th17 cells by flow cytometry from OHCA patients after ROSC on days 1 and 3 and from healthy individuals.

Results: Compared with healthy individuals, T lymphocyte counts and Th1 cell counts decreased on days 1 and 3 after ROSC (1464 [1198, 2152] vs. 779 [481, 1140] vs. 581 [324, 1118]/μl, χ^2 = 30.342, P < 0.001; 154 [90, 246] vs. 39 [19, 78] vs. 24 [12, 53]/μl, χ^2 = 42.880, P < 0.001), and Th2 and Th17 cell counts decreased on day 3 (17.0 [10.8, 24.0] vs. 9.0 [3.0, 15.5]/μl, Z = –3.228, P = 0.001; 4.7 [2.7, 9.1] vs. 2.7 [1.0, 6.5]/μl, Z = –2.294, P = 0.022). No change in CD4+/CD3+ lymphocyte ratio was seen on day 1 or day 3 (57.9 [49.4, 63.0] vs. 55.4 [46.5, 66.5] vs. 55.4 [50.2, 67.0]%, χ^2 = 0.171, P = 0.918). Th1/CD4+ lymphocyte ratio decreased on days 1 and 3 (19.0 [14.0, 24.9] vs. 9.3 [4.6, 13.9] vs. 9.5 [4.9, 13.6]%, χ^2 = 25.754, P < 0.001), and Th2/CD4+ lymphocyte ratio increased on day 1 and decreased on day 3 (1.9 [1.2, 2.5] vs. 2.5 [1.6, 4.0] vs. 1.9 [1.6, 3.8]%, χ^2 = 6.913, P = 0.032). Th1/Th2 cell ratio also decreased on both days (9.4 [7.3, 13.5] vs. 3.1 [1.9, 5.6] vs. 4.2 [2.8, 5.9], χ^2 = 44.262, P < 0.001). Despite an upward trend in the median of Th17/CD4+ lymphocyte ratio in OHCA patients, there was no significant difference compared with healthy individuals (0.9 [0.4, 1.2] vs. 0.7 [0.4, 1.2] vs. 0.6 [0.3, 1.0]%, χ^2 = 2.620, P = 0.270). The dynamic expression of Th1/Th2/Th17 cells on days 1 and 3 were simultaneously analyzed in 28/53 OHCA patients who survived >3 days; patients were divided into survivors (n = 10) and nonsurvivors (n = 18) based on 28-day survival. No significant differences in Th1/Th2/Th17 cell counts, ratios in CD4+ lymphocytes, and Th1/Th2 cell ratio were seen between survivors and nonsurvivors on both days (all P > 0.05). There was no difference over time in both survivors and nonsurvivors (all P > 0.05).

Conclusion: Downregulated T lymphocyte counts, including Th1/Th2/Th17 subsets and Th1/Th2 cell ratio imbalance, occur in the early period after ROSC, that may be involved in immune dysfunction in OHCA patients.

Key words: Out-of-Hospital Cardiac Arrest; T Helper Type 1 Cell; T Helper Type 17 Cell; T Helper Type 2 Cell

INTRODUCTION

Out-of-hospital cardiac arrest (OHCA) results in severe shock, and the interruption of blood flow leads to disruption in the delivery of oxygen and metabolic substrates, which affects every system including the immune system. After the return of spontaneous circulation (ROSC), OHCA patients present with a "sepsis-like" syndrome, which is characteristic of the high levels of circulating cytokines and the presence of endotoxin in plasma.[1,2] *In vitro*, the cytokine production and proliferation abilities of circulatory lymphocytes of OHCA patients were impaired.[2-4] This is similar to lymphocytes

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observed in sepsis.[5] After ROSC, OHCA patients are prone to infection, and infections occur in more than 2 out of 3 OHCA patients after successful resuscitation during hospitalization.[6-8] Therefore, immune dysregulation is an important feature of OHCA patients after ROSC.

Modulation of the blood immune response is an important feature following ischemia/reperfusion injury,[9] and T lymphocytes, primarily CD4+ and CD8+, play an important role in organ ischemia reperfusion injury.[10] CD4+ T cells can be further divided into different subsets by their phenotypes and functions. In addition to the classical differentiation of T-helper type (Th) 1 and Th2 cells, Th17, Th9, T follicular-helper, and T-regulatory cells have been recognized. T-helper subsets are crucial for directing appropriate immune responses during host defense and have been extensively studied in infection and immune-mediated inflammatory diseases.[11-15] In previous cardiac arrest/cardiopulmonary resuscitation porcine model studies, an imbalance of Th1/Th2 cells was observed in splenic, lung, and myocardial tissue.[16-18] Serum interferon gamma (IFN-γ)/interleukin 4 (IL-4), which are secreted by Th1/Th2 cells and involved in the differentiation of naive T lymphocytes into Th1/Th2 cells, presented increased IL-4 and decreased IFN-γ levels. IL-10, secreted by Th2 cells, was also found to be elevated in OHCA patients and animal studies.[2,19] The above studies indicate a potential imbalance of Th1/Th2 in the circulation. Nevertheless, to date, there have been few clinical reports regarding T-helper subsets in OHCA patients. Therefore, in this study, we observed the changes in blood T-helper subsets including Th1, Th2, and Th17 cells, to explore the early immune changes in OHCA patients after ROSC.

Methods

Ethical approval

This study was approved by the local Medical Ethics Committees of the participating hospitals, including Beijing Chao-Yang Hospital, Beijing Shijingshan Hospital, Beijing Luhe Hospital, and Beijing Friendship Hospital, and was in accordance with the *Helsinki Declaration* of 1975, as revised in 2000. The requirement for informed consent was waived because this study was observational, and biomarker expression was measured on residual blood after completion of routine tests every morning.

Study participants

During July–September 2016 and March–September 2017, OHCA patients admitted to the emergency departments of participating hospitals were evaluated for potential enrollment. According to the 2015 International Consensus on Cardiopulmonary Resuscitation, rescuers should begin cardiopulmonary resuscitation if an adult is unresponsive and not breathing normally (disregarding occasional gasps).[20] The inclusion criteria were as follows: ROSC >12 h and Glasgow Coma Scale (GCS) score <8 after ROSC. The exclusion criteria were as

follows: age <18 years, obvious infection of any organ or tissue, terminal stage of disease, and immunosuppressive therapy in the last 3 months. All patients were treated according to the 2015 International Consensus on Cardiopulmonary Resuscitation.[21] In addition, healthy individuals, approximately matched by age and sex, who underwent health physical examination, were enrolled in this study.

Data collection

Participant data, including demographics; resuscitation data, including prehospital adrenaline dose, initial cardiac rhythm, and time to ROSC; and clinical and laboratory data were recorded. Acute Physiology and Chronic Health Evaluation II (APACHE II) and the Sequential Organ Failure Assessment (SOFA) scores were calculated to assess severity. The expression of Th1, Th2, and Th17 cells in residual heparin lithium anticoagulant blood samples, after completion of routine clinical tests every morning or health physical examination, were analyzed. In this study, blood samples were collected on day 1 (22.7 ± 7.9 h after OHCA) and day 3 (71.1 ± 12.1 h after OHCA). During follow-up, the survival outcomes after 28 days were collected. The flowchart of the participant selection and analysis for this study is shown in Figure 1.

Isolation of peripheral lymphocytes and incubation

Peripheral blood mononuclear cells (PBMCs) were isolated through differential centrifugation by lymphocyte separation medium (Haoyang, Tianjin, China) from whole blood within 2 h of collection. Then, $3–5 \times 10^5$ PBMCs were suspended in 500 µl of RPMI-1640 medium (Corning, Manassas, USA) supplemented with 10% fetal bovine serum (Sijiqing, Zhejiang, China). One microliter of the mixture of ionomycin and Brefeldin A (BD Bioscience, San Diego, USA) was added to the medium, and lymphocytes were incubated in a humidified incubator at 37°C with an atmosphere comprising 5% CO_2 for 260 min.

Flow cytometry
Determination of Th1/Th2/Th17

After incubation, surface staining for CD3 and CD8 was performed for 20 min in a dark area, and PBMCs were washed with stain buffer (BD Bioscience). Then, PBMCs were fixed at 4°C for 40 min and washed with Perm/Wash Buffer (BD Bioscience), followed by intracellular staining for IFN-γ + (Th1), IL-4 (Th2), and IL-17 (Th17) at 4°C for 40 min. According to the manufacturer's recommendations, the following monoclonal antibodies and their isotype controls were used: APC-H7 labeled anti-CD3 (2 µl, clone SK7; BD Bioscience), eFluor® 450-labeled anti-CD8 (2 µl, clone RPA-T8; eBioscience, San Jose, USA), PE-Cy7 labeled anti-IFN-γ (2 µl, BD Bioscience), APC-labeled anti-IL-4 (2 µl, BD Bioscience), and PE-labeled anti-IL-17 (5 µl, BD Bioscience). Finally, PBMCs were washed with Perm/Wash Buffer.

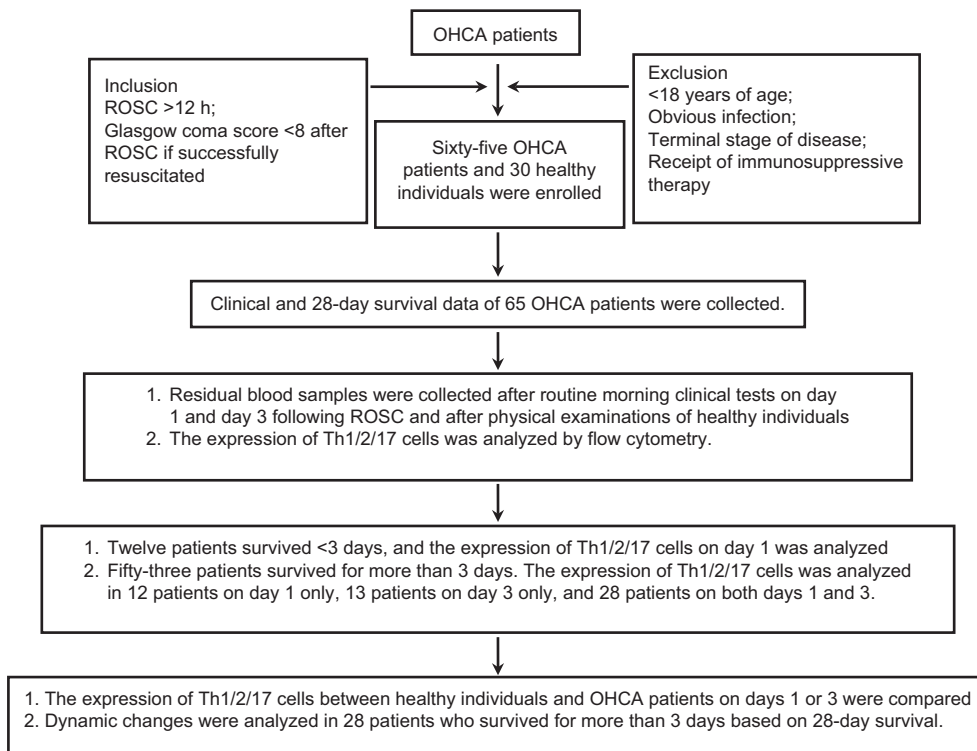The sample measurements were performed using the Gallios™ Flow Cytometer (Beckman Coulter, Inc.)

Figure 1: Flowchart of participant selection and analysis in this study. OHCA: Out-of-hospital cardiac arrest; ROSC: Return of spontaneous circulation; Th: T helper type.
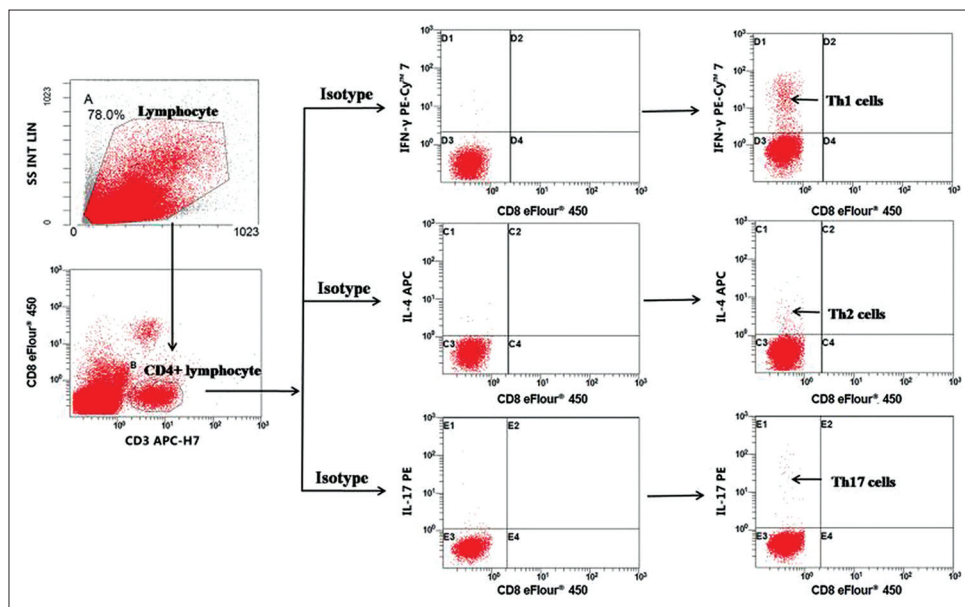

Figure 2: Representative flow dot plots of the expression of Th1, Th2, and Th17 cells. Th: T helper type.

and analyzed with Gallios Software Version 1.0. The lymphocytes were gated by forward scatter and side scatter. For the determination of Th1/Th2/Th17 cells, CD4+ T cells were gated as CD3+ CD8− cells [Figure 2]. Th1, Th2, and Th17 cells were gated as CD3+ CD8− IFN-γ+, CD3+ CD8− IL-4+, and CD3+ CD8− IL-17+ cells, respectively. The threshold was defined using an isotype control. The results are expressed as the percentage in CD4+ cells.

T lymphocyte counts

Absolute CD3+ lymphocyte counts were obtained using Flow-Count Fluorospheres (Beckman Coulter, Inc.) according to the manufacturer's instructions. Approximately 50 μl of peripheral blood sample was stained with Pacific Blue-labeled anti-CD3 (1 μl, Clone UCHT1; BD Bioscience) and FITC labeled anti-CD4 (1 μl, Clone OKT4; eBioscience) for 20 min in a dark area. Erythrocytes were lysed for 15 min, and the cells were washed twice. Then, CD4+/CD3+ lymphocyte

ratios and CD3+ CD4+ lymphocyte counts were calculated. Th1, Th2, and Th17 cell counts were calculated by multiplying CD3+CD4+ counts by their ratios in CD3+CD4+ lymphocytes.

Statistical analysis

All data were analyzed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA). For normally distributed data, continuous variables were presented as mean ± standard deviation. For data with skewed distributions, variables were expressed as median (25th and 75th percentiles). The Kruskal–Wallis test was applied for multigroup comparisons, and the Mann–Whitney U-test was performed for two-group comparisons. The Wilcoxon-signed rank test was performed for paired sample comparisons. Qualitative parameters were analyzed using a 2×2 contingency table followed by the Chi-square test. The correlation was analyzed using Spearman's rank correlation. All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

Sixty-five OHCA patients and 30 healthy individuals were enrolled in the study. Demographic and clinical characteristics are presented in Table 1. In this study, acute cardiac and cerebral events were the main causes of OHCA, and other causes of OHCA including poisoning, hypokalemia, and unexplained causes. Of the 65 OHCA patients, 12 patients who survived for <3 days were analyzed for the expression of Th1/Th2/Th17 cells on day 1 only. Of the 53 patients who survived for greater than 3 days, the expression of Th1/Th2/Th17 cells were analyzed in 12 patients on day 1 only, 13 patients on day 3 only, and 28 patients on both day 1 and day 3. Dynamic changes were analyzed in these 28 patients. According to 28-day survival, the 28 OHCA patients whose blood samples were analyzed on day 1 and day 3 were divided into survivors and nonsurvivors, and the characteristics of patients and initial resuscitation data are shown in Table 2.

Changes in T lymphocyte counts in out-of-hospital cardiac arrest patients during the first 3 days after return of spontaneous circulation

Compared with healthy individuals, circulatory CD3+ T lymphocyte counts decreased on day 1 after ROSC and maintained at a low level on day 3 (1464 [1198, 2152] vs. 779 [481, 1140] vs. 581 [324, 1118]/μl, $\chi^2 = 30.342$, $P < 0.001$). The ratios of CD4+/CD3+ lymphocytes showed no change (0.58 [0.49, 0.63] vs. 0.55 [0.47, 0.67] vs. 0.55 [0.50, 0.67], $\chi^2 = 0.171$, $P = 0.918$). However, CD3 + CD4+ lymphocyte counts significantly decreased on day 1, without further decrease on day 3 (875 [601, 1228] vs. 447 [281, 690] vs. 359 [181, 620]/μl, $\chi^2 = 27.274$, $P < 0.001$). On day 1, no

Table 1: OHCA patient characteristics on admission

Variables	Healthy control group ($n = 30$)	OHCA group ($n = 65$)	Statistical values	P
Age (years)	66.5 (58.0, 73.0)	71.0 (56.0, 79.5)	−1.105*	0.269
Male/female (n/n)	20/10	38/27	0.581†	0.446
Previous medical history, n (%)				
Hypertension	7 (23.3)	28 (43.1)	3.349†	0.064
Diabetes	4 (13.3)	26 (40)	6.756†	0.009
Coronary heart disease	6 (20.0)	11 (16.9)	0.132†	0.716
Asthma or COPD	3 (10.0)	5 (7.7)	0.000‡	1.000
Deep venous thrombosis	0	3 (4.6)	–	0.549§
Dilated cardiomyopathy	0	1 (1.5)	–	1.000§
Cardiac arrest cause, n (%)				
Acute myocardial infarction		24 (36.9)		
Acute heart failure		16 (24.6)		
Asphyxia or acute respiratory failure		7 (10.8)		
Cerebral hemorrhage/infarction		3 (4.6)		
Craniocerebral trauma		4 (6.2)		
Acute pulmonary embolism		3 (4.6)		
Others		8 (12.3)		
Initial resuscitation				
Time to ROSC (min)		20.0 (10.0, 26.0)		
Epinephrine (mg)		3.0 (1.5, 5.0)		
Initial cardiac rhythm, n (%)				
Ventricular arrhythmia		13 (20.0)		
Asystole and pulseless activity		52 (80.0)		
APACHE II score		42.0 (39.5, 45.0)		
SOFA score		13.0 (12.0, 14.0)		
Day 28 mortality, n (%)		42 (64.6)		

Data are shown as median (interquartile range) or n (%). *Z value; †Chi-square value; ‡Continuity correction; §When the expectations of two cells are <1, Fisher's exact test was used. APACHE II: Acute Physiology and Chronic Health Evaluation II; COPD: Chronic obstructive pulmonary disease; ROSC: Return of spontaneous circulation; OHCA: Out-of-hospital cardiac arrest; SOFA: Sequential Organ Failure Assessment.

Table 2: Characteristics of the 28 OHCA patients on admission based on 28-day survival

Variables	Survivors ($n = 10$)	Nonsurvivors ($n = 18$)	Statistical values	P
Age (years)	66.0 (48.3, 79.0)	66.5 (49.0, 81.0)	−0.264*	0.792
Male/female	6/4	8/10	0.622†	0.430
Initial resuscitation				
Time to ROSC (min)	15.0 (13.8, 23.3)	20.0 (10.0, 31.8)	−0.772*	0.464
Epinephrine (mg)	2.5 (1.0, 3.5)	4.0 (2.0, 5.0)	−1.388*	0.175
Initial cardiac rhythm				
Ventricular arrhythmia	3	1	1.458‡	0.227
Asystole and pulseless activity	7	17	1.458‡	0.227
APACHE II score	42.0 (39.0, 45.3)	42.5 (38.8, 44.8)	−0.048*	0.981
SOFA score	12.0 (12.0, 13.0)	13.0 (12.0, 14.5)	−1.351*	0.226

Data are shown as median (interquartile range) or *n*. *Z value; †Chi-square value; ‡Continuity correction. APACHE II: Acute Physiology and Chronic Health Evaluation II; OHCA: Out-of-hospital cardiac arrest; ROSC: Return of spontaneous circulation; SOFA: Sequential Organ Failure Assessment.

difference in CD3+ and CD3+ CD4+ lymphocyte counts or CD4+/CD3+ lymphocyte ratios were found between survivors and nonsurvivors.

Changes in circulatory Th1 cells in out-of-hospital cardiac arrest patients in the first 3 days after return of spontaneous circulation

On day 1 after ROSC, both Th1/CD4+ lymphocyte ratios and circulatory Th1 cell counts decreased and were maintained at a low level on day 3 [Figure 3a and 3b]. In this study, dynamic expression of both Th1/CD4+ lymphocyte ratios and Th1 cell counts in 28 OHCA patients were analyzed. No significant difference was found between day 1 and day 3 in both survivors and nonsurvivors [Figure 3c and 3d]. Meanwhile, the results showed no difference between survivors and nonsurvivors on days 1 and 3.

Changes in circulatory Th2 cells in out-of-hospital cardiac arrest patients in the first 3 days after return of spontaneous circulation

Compared with healthy individuals, Th2/CD4+ lymphocyte ratios were elevated on day 1 ($Z = -2.751$, $P = 0.006$) and began to decline on day 3 ($Z = -1.393$, $P = 0.164$) [Figure 4a]. Circulatory Th2 lymphocyte counts showed a downward trend on day 1 ($Z = -1.822$, $P = 0.069$) and further decreased on day 3 ($Z = -3.228$, $P < 0.001$) [Figure 4b]. Both Th2/CD4+ lymphocyte ratios and circulatory Th2 lymphocyte counts showed no significant difference between survivors and nonsurvivors on days 1 and 3. No dynamic changes in Th2/CD4+ lymphocyte ratios and circulatory Th2 lymphocyte counts were seen in both survivors and nonsurvivors [Figure 4c and 4d].

Changes in Th17 cells in out-of-hospital cardiac arrest patients in the first 3 days after return of spontaneous circulation

Compared with healthy individuals, Th17/CD4+ lymphocyte ratios showed an upward trend; however, no significant difference was found on days 1 ($Z = -1.492$, $P = 0.136$) and 3 ($Z = -1.393$, $P = 0.170$) [Figure 5a].

Circulatory Th17 lymphocyte counts showed a downward trend on day 1 ($Z = -1.724$, $P = 0.085$), and further decreased on day 3 ($Z = -2.294$, $P = 0.022$) [Figure 5b]. No dynamic changes in Th17/CD4+ lymphocyte ratios and Th17 lymphocyte counts were seen between days 1 and 3 in both survivors and nonsurvivors [Figure 5c and 5d]. Meanwhile, both showed no significant differences between survivors and nonsurvivors on days 1 and 3.

Changes in circulatory Th1/Th2 ratio in out-of-hospital cardiac arrest patients in the first three days after return of spontaneous circulation

Compared with healthy individuals, the Th1/Th2 ratio significantly decreased on day 1, without further decrease on day 3 (9.40 [7.33, 13.48] vs. 3.05 [1.85, 5.55] vs. 4.20 [2.80, 5.85], $\chi^2 = 44.262$, $P < 0.001$). On day 1, no significant difference was seen between survivors and nonsurvivors (3.70 [2.30, 6.10] vs. 2.60 [1.75, 4.45], $Z = -1.093$, $P = 0.274$). Meanwhile, no changes with time were seen in both survivors and nonsurvivors.

DISCUSSION

After ROSC, the immune dysfunction due to systemic ischemia-reperfusion is an important feature of OHCA patients. In view of the immune regulatory role of CD4+ lymphocytes, we explored the early changes in T lymphocyte subsets. The present results showed decreased circulatory Th1, Th2, and Th17 cell counts and an imbalance of Th1/Th2 cells in the circulation of OHCA patients after ROSC.

In this study, the total T lymphocyte count and subsets, including Th1, Th2, and Th17 cell counts, of OHCA patients decreased in the first 3 days after ROSC. Different changes were seen in the T lymphocyte subsets. First, the Th1/CD4+ lymphocyte ratio decreased on days 1 and 3. Second, the Th2/CD4+ lymphocyte ratio increased on day 1 and declined from its peak value on day 3. Third, the Th1/Th2 cell ratio significantly decreased on days 1 and 3. Fourth, despite the slightly elevated Th17/CD4+ lymphocyte ratio seen in the OHCA group, this difference was not statistically
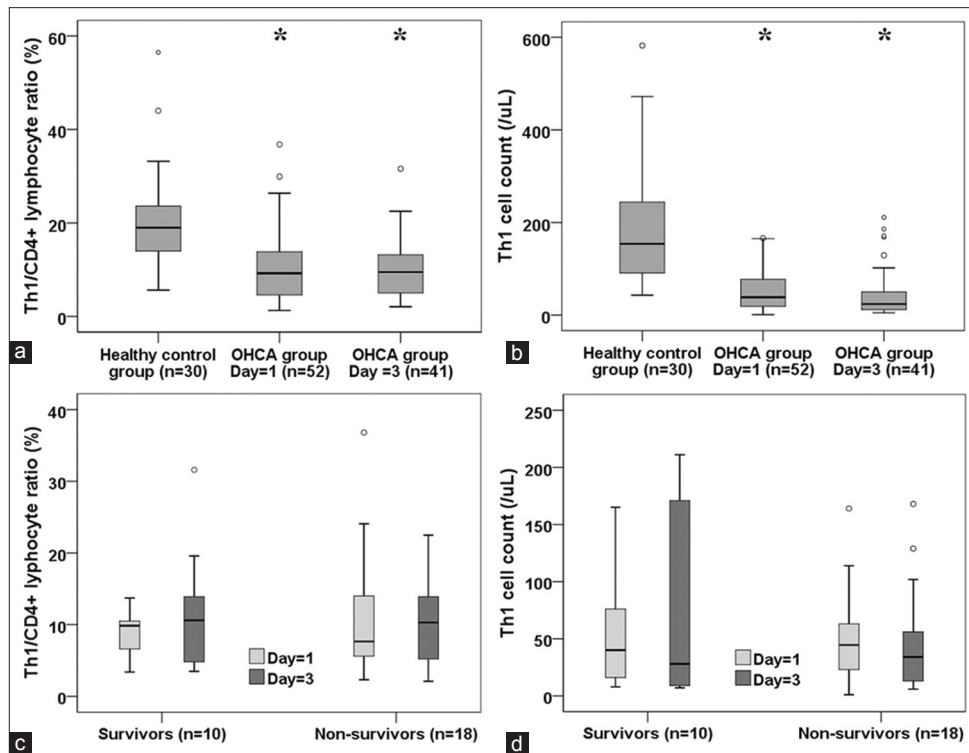
Figure 3: Changes in Th1 cells in OHCA patients after ROSC. (a and b) Both Th1/CD4+ lymphocyte ratio and Th1 cell counts decreased on days 1 and 3 after ROSC. (c and d) Dynamic observation in 28 patients revealed no significant differences in both Th1/CD4+ lymphocyte ratio and Th1 cell counts between days 1 and 3 in both survivors and nonsurvivors. Meanwhile, no statistical difference was found between survivors and nonsurvivors on days 1 and 3. *$P < 0.001$ compared with the healthy control group. OHCA: Out-of-hospital cardiac arrest; ROSC: Return of spontaneous circulation; Th: T helper type.
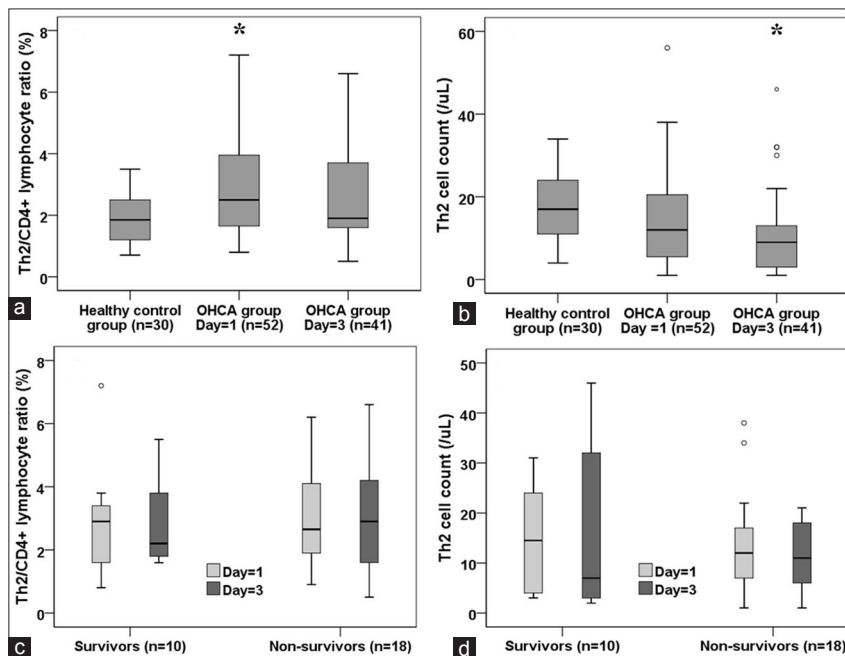

Figure 4: Changes in Th2 cells in OHCA patients after ROSC. (a) Compared with healthy individuals, Th2/CD4+ lymphocyte ratio increased on day 1 and showed a downward trend on day 3. (b) Th2 cell counts showed a downward trend. (c and d) Both Th2/CD4+ lymphocyte ratio and Th2 cell counts showed no significant change between days 1 and 3 in survivors and nonsurvivors. No statistical difference was shown between survivors and nonsurvivors on days 1 and 3. *$P < 0.01$ compared with healthy control group. OHCA: Out-of-hospital cardiac arrest; ROSC: Return of spontaneous circulation; Th: T helper type.

different compared with that of healthy individuals. Finally, we did not find any difference in the above T lymphocyte subset ratios and counts between survivors and nonsurvivors on days 1 and 3.
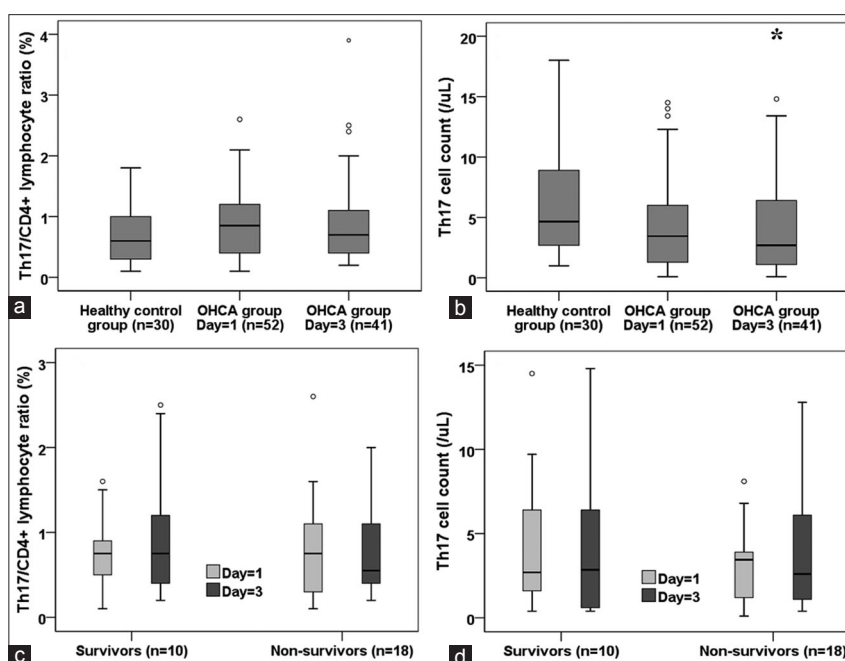
Figure 5: Changes in Th17 cells in OHCA patients after ROSC. (a) Compared with healthy individuals, no difference of Th17/CD4+ lymphocyte ratio was found on days 1 and 3 after ROSC. (b) Th17 cell counts decreased on day 3. (c and d) Both Th17/CD4+ lymphocyte ratio and Th17 cell counts showed no significant differences between days 1 and 3 in survivors and nonsurvivors. No statistical difference was found between survivors and nonsurvivors on days 1 and 3. *$P < 0.05$ compared with healthy control group. OHCA: out-of-hospital cardiac arrest; ROSC: return of spontaneous circulation; Th: T helper type.

The balance between Th1 and Th2 cells is important in regulating immune function and the inflammatory response. Th1 cells produce IFN-γ and IL-2, favoring cell-mediated immunity, and Th2 lymphocytes secrete IL-4, IL-5, and IL-10, favoring humoral immunity.[22,23] In this study, we determined Th1 and Th2 cells by their intracellular expression of IFN-γ and IL-4. IFN-γ, a key cytokine secreted by Th1 cells, can promote naïve T lymphocytes to differentiate into Th1 cells, and inhibit the proliferation of Th2 cells. IL-4 can stimulate and activate the proliferation of B and T lymphocytes, promote the differentiation of naïve T lymphocytes into Th2 cells, and inhibit the differentiation of naïve T lymphocytes into Th1 cells. The present results showed that both Th1 cell count and Th1/CD4+ lymphocyte ratio decreased in the first 3 days after ROSC. This result is consistent with previous studies that IFN-γ produced by the T lymphocytes of OHCA patients was significantly reduced[2] and also supports that OHCA patients have impaired cell-mediated immunity.[4] Comparatively, despite the decreased Th2 cell counts, the Th2/CD4+ lymphocyte ratio had a relative increase on day 1. Th2 cells not only favor humoral immunity but also produce IL-4 and IL-10, which can suppress cell-mediated immunity and systematic immunity.[24-26] The results are consistent with the transiently elevated expression of IL-10 observed in OHCA patients and the cardiac arrest animal model.[2,19] Thus, the relatively elevated Th2 cell ratio in the early period after ROSC may negatively affect the immunity of OHCA patients.

Th1 cells are crucial in the host defense against intracellular pathogens whereas Th2 cells are known to be important for the elimination of helminthic parasites.[27,28] Thus, the Th1/Th2 cell balance is useful for classifying immune responses that occur in the elimination of microbial pathogens. In this study, a consistently decreased Th1/Th2 ratio was seen in OHCA patients after ROSC. An imbalance in the Th1/Th2 cell ratio is found in septic patients.[29] In these patients, there is an obvious shift from a Th1 response to a Th2 response, which results in patients who are more vulnerable to infections.[30] In other words, patients with low Th1/Th2 cell ratios develop infections more easily. Therefore, decreased Th1/Th2 cell ratios in the early period after ROSC may contribute to the high rate of infections found in OHCA patients during hospitalization.[6-8]

We also observed changes in Th17 cells. Th17 cells have recently been identified as a unique CD4+ T-helper cell subset, characterized by IL-17 production that promotes tissue inflammation.[31,32] In this study, similar to Th1 and Th2 cells, circulatory Th17 cell counts also decreased. Although no difference was observed compared with those of healthy individuals, a slight upward trend in the circulatory Th17/CD4+ lymphocyte ratios of OHCA patients was shown. This result may imply that circulatory Th17 cells are involved in the systemic inflammatory response of OHCA patients, similar to that found in sepsis and burn injury.[14,15] Th17 cells account for a small proportion of blood lymphocytes; therefore, a larger number of samples are needed to evaluate the expression of circulatory Th17 cells. Moreover, its major immunomodulatory secretory products, IL-17 and IL-22, need be monitored to further evaluate the impact of Th17 cells in future studies.

We did not find any difference in Th1, Th2, and Th17 lymphocyte counts between survivors and nonsurvivors. This can be explained by the similar time to ROSC and APACHE II and SOFA scores of both groups, which indicate systemic ischemic hypoxia. It is possible that different degrees of systemic ischemic hypoxia lead to different degrees of change. We also analyzed dynamic changes in the above indicators in OHCA patients who survived for more than 3 days and found no significant between changes on days 1 and 3. This implies that persistent immune dysfunction may exist in OHCA patients in the early period after ROSC.

Impaired cell immunity was found in OHCA patients,[4] and an imbalance in the Th1/Th2 cell ratio in the early period after ROSC was associated with impaired cell immunity, which may make patients more prone to infection. Hospital-acquired infections significantly extend the length of the Intensive Care Unit and hospital-stay after OHCA.[33,34] Therefore, timely clinical intervention by doctors may be necessary to protect patients from infection in future studies. At present, prophylactic antibiotics are commonly prescribed for OHCA patients after ROSC. Whether promoting cell immunity is more effective than prophylactic antibiotics for the prevention of hospital-acquired infection is unknown. The present study provides theoretical evidence for future studies.

This study has some limitations. First, due to clinical complexities, such as fever of central origin/therapeutic hypothermia, the impact of stress on white blood cells, and interobserver agreement in the interpretation of chest radiographs, the exact affirmation of infection is difficult.[33,35] Therefore, we did not analyze the correlation between T lymphocyte subsets and infection and only emphasized early changes in T lymphocyte subsets. Second, in order to assess obvious changes, we only enrolled OHCA patients who had obvious signs of systemic ischemic hypoxia, such as GCS <8 after ROSC. The changes in T lymphocyte subsets in patients with mild systemic ischemic hypoxia need to be further studied. Third, because the accurate no-flow time of most patients could not be assessed, we did not analyze the impact of no-flow time.

In conclusion, circulatory T lymphocyte levels, including Th1, Th2, and Th17 cell counts, decrease in the early period after ROSC. Despite the unchanged CD4+/CD3+ lymphocyte ratio, a shift from a Th1 to Th2 response occurs in the circulation. The changes in Th1, Th2, and Th17 subsets may contribute to immune regulatory dysfunction in OHCA patients in the early period after ROSC.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Grimaldi D, Sauneuf B, Guivarch E, Ricome S, Geri G, Charpentier J, et al. High level of endotoxemia following out-of-hospital cardiac arrest is associated with severity and duration of postcardiac arrest shock. Crit Care Med 2015;43:2597-604. doi: 10.1097/CCM.0000000000001303.
2. Adrie C, Adib-Conquy M, Laurent I, Monchi M, Vinsonneau C, Fitting C, et al. Successful cardiopulmonary resuscitation after cardiac arrest as a "sepsis-like" syndrome. Circulation 2002;106:562-8. doi: 10.1161/01.CIR.0000023891.80661.AD.
3. Beurskens CJ, Horn J, de Boer AM, Schultz MJ, van Leeuwen EM, Vroom MB, et al. Cardiac arrest patients have an impaired immune response, which is not influenced by induced hypothermia. Crit Care 2014;18:R162. doi: 10.1186/cc14002.
4. Soppi E, Lindroos M, Nikoskelainen J, Kalliomäki JL. Effect of cardiopulmonary resuscitation-induced stress on cell-mediated immunity. Intensive Care Med 1984;10:287-92. doi: 10.1007/BF00254317.
5. Venet F, Lukaszewicz AC, Payen D, Hotchkiss R, Monneret G. Monitoring the immune response in sepsis: A rational approach to administration of immunoadjuvant therapies. Curr Opin Immunol 2013;25:477-83. doi: 10.1016/j.coi.2013.05.006.
6. Kuchena A, Merkel MJ, Hutchens MP. Postcardiac arrest temperature management: Infectious risks. Curr Opin Crit Care 2014;20:507-15. doi: 10.1097/MCC.0000000000000125.
7. Mongardon N, Perbet S, Lemiale V, Dumas F, Poupet H, Charpentier J, et al. Infectious complications in out-of-hospital cardiac arrest patients in the therapeutic hypothermia era. Crit Care Med 2011;39:1359-64. doi: 10.1097/CCM.0b013e3182120b56.
8. Tsai MS, Chiang WC, Lee CC, Hsieh CC, Ko PC, Hsu CY, et al. Infections in the survivors of out-of-hospital cardiac arrest in the first 7 days. Intensive Care Med 2005;31:621-6. doi: 10.1007/s00134-005-2612-6.
9. Boros P, Bromberg JS. New cellular and molecular immune pathways in ischemia/reperfusion injury. Am J Transplant 2006;6:652-8. doi: 10.1111/j.1600-6143.2005.01228.x.
10. Rao J, Lu L, Zhai Y. T cells in organ ischemia reperfusion injury. Curr Opin Organ Transplant 2014;19:115-20. doi: 10.1097/MOT.0000000000000064.
11. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. Cytokine 2015;74:5-17. doi: 10.1016/j.cyto.2014.09.011.
12. Li J, Li M, Su L, Wang H, Xiao K, Deng J, et al. Alterations of T helper lymphocyte subpopulations in sepsis, severe sepsis, and septic shock: A prospective observational study. Inflammation 2015;38:995-1002. doi: 10.1007/s10753-014-0063-3.
13. Wu HP, Chung K, Lin CY, Jiang BY, Chuang DY, Liu YC, et al. Associations of T helper 1, 2, 17 and regulatory T lymphocytes with mortality in severe sepsis. Inflamm Res 2013;62:751-63. doi: 10.1007/s00011-013-0630-3.
14. Brunialti MK, Santos MC, Rigato O, Machado FR, Silva E, Salomao R, et al. Increased percentages of T helper cells producing IL-17 and monocytes expressing markers of alternative activation in patients with sepsis. PLoS One 2012;7:e37393. doi: 10.1371/journal.pone.0037393.
15. Rendon JL, Choudhry MA. Th17 cells: Critical mediators of host responses to burn injury and sepsis. J Leukoc Biol 2012;92:529-38. doi: 10.1189/jlb.0212083.
16. Gu W, Zhang Q, Li CS. Effect of splenic regulatory T-cell apoptosis on the postresuscitation immune dysfunction in a porcine model. Chin Med J 2016;129:1577-83. doi: 10.4103/0366-6999.184461.
17. Gu W, Li CS, Yin WP, Hou XM, Zhang J, Zhang D, et al. Expression imbalance of transcription factors GATA-3 and T-bet in post-resuscitation myocardial immune dysfunction in a porcine model of cardiac arrest. Resuscitation 2013;84:848-53. doi: 10.1016/j.resuscitation.2012.11.023.
18. Gu W, Li C, Yin W, Hou X, Zhang D. Effects of shen-fu injection on the expression of T-cell-specific transcription factors T-bet/Gata-3 in porcine postresuscitation lung injury. Evid Based Complement Alternat Med 2013;2013:464650. doi: 10.1155/2013/464650.
19. Callaway CW, Rittenberger JC, Logue ES, McMichael MJ.

Hypothermia after cardiac arrest does not alter serum inflammatory markers. Crit Care Med 2008;36:2607-12. doi: 10.1097/CCM.0b013e318184443b.

20. Perkins GD, Travers AH, Berg RA, Castren M, Considine J, Escalante R, *et al.* Part 3: Adult basic life support and automated external defibrillation: 2015 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. Resuscitation 2015;95:e43-69. doi: 10.1016/j.resuscitation.2015.07.041.

21. Soar J, Callaway CW, Aibiki M, Böttiger BW, Brooks SC, Deakin CD, *et al.* Part 4: Advanced life support: 2015 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. Resuscitation 2015;95:e71-120. doi: 10.1016/j.resuscitation.2015.07.042.

22. Bretscher PA. On the mechanism determining the TH1/TH2 phenotype of an immune response, and its pertinence to strategies for the prevention, and treatment, of certain infectious diseases. Scand J Immunol 2014;79:361-76. doi: 10.1111/sji.12175.

23. Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? Nat Rev Immunol 2010;10:225-35. doi: 10.1038/nri2735.

24. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, *et al.* The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006;126:1121-33. doi: 10.1016/j.cell.2006.07.035.

25. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;75:263-74. doi: 10.1016/0092-8674(93)80068-p.

26. Leon LR, Kozak W, Kluger MJ. Role of IL-10 in inflammation. Studies using cytokine knockout mice. Ann N Y Acad Sci 1998;856:69-75. doi: 10.1111/j.1749-6632.1998.tb08314.x.

27. Reiner SL, Locksley RM. The regulation of immunity to Leishmania major. Annu Rev Immunol 1995;13:151-77. doi: 10.1146/annurev.iy.13.040195.001055.

28. Pulendran B, Artis D. New paradigms in type 2 immunity. Science 2012;337:431-5. doi: 10.1126/science.1221064.

29. Ferguson NR, Galley HF, Webster NR. T helper cell subset ratios in patients with severe sepsis. Intensive Care Med 1999;25:106-9. doi: 10.1007/s001340050795.

30. Tan M, Zhu JC, Du J, Zhang LM, Yin HH. Effects of probiotics on serum levels of th1/Th2 cytokine and clinical outcomes in severe traumatic brain-injured patients: A prospective randomized pilot study. Crit Care 2011;15:R290. doi: 10.1186/cc10579.

31. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, *et al.* Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6:1123-32. doi: 10.1038/ni1254.

32. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, *et al.* A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol 2005;6:1133-41. doi: 10.1038/ni1261.

33. Hellenkamp K, Onimischewski S, Kruppa J, Faßhauer M, Becker A, Eiffert H, *et al.* Early pneumonia and timing of antibiotic therapy in patients after nontraumatic out-of-hospital cardiac arrest. Crit Care 2016;20:31. doi: 10.1186/s13054-016-1191-y.

34. Perbet S, Mongardon N, Dumas F, Bruel C, Lemiale V, Mourvillier B, *et al.* Early-onset pneumonia after cardiac arrest: Characteristics, risk factors and influence on prognosis. Am J Respir Crit Care Med 2011;184:1048-54. doi: 10.1164/rccm.201102-0331OC.

35. Hopstaken RM, Witbraad T, van Engelshoven JM, Dinant GJ. Inter-observer variation in the interpretation of chest radiographs for pneumonia in community-acquired lower respiratory tract infections. Clin Radiol 2004;59:743-52. doi: 10.1016/j.crad.2004.01.011.

院外心跳骤停患者心肺复苏成功后早期阶段循环中 Th1、Th2及Th17细胞的变化

摘要

背景：免疫紊乱是院外心跳骤停（out-of-hospital cardiac arrest，OHCA）患者恢复自主循环（return of spontaneous circulation，ROSC）后的一个重要特征。在本研究中，我们观察了循环辅助性T细胞（T helper type 1，Th1）、Th2及Th17细胞的表达，以探讨OHCA患者ROSC后早期免疫功能改变。

方法：从2016年7月至9月及2017年3月至9月，共收集65名恢复自主循环超过12小时的OHCA患者及30名健康对照者，收集患者临床及28天生存数据。通过流式细胞术分析OHCA患者恢复自主循环后第1、3天及健康对照者外周血中Th1、Th2及Th17细胞的表达。

结果：与健康对照组相比，在ROSC后的第1天及3天，T淋巴细胞及Th1细胞计数降低，在第3天Th2及Th17细胞计数降低。在第1天及第3天，CD4+/CD3+淋巴细胞比例无明显改变。尽管如此，在第1天及第3天Th1/CD4+淋巴细胞比例降低，Th2/CD4+淋巴细胞比例在第1天升高，第3天开始下降。Th1/Th2比例在第1天及第3天同样降低。尽管在OHCA患者Th17/CD4+淋巴细胞比例呈现上升趋势，但与健康对照比较无统计学差异。53名存活超过3天的患者中，有28名患者同时分析了第1天及第3天Th1、Th2及Th17细胞的动态表达，按照28天的生存结局分为生存者（n=10）及非生存者（n=18）。在第1天及第3天，Th1、Th2及Th17细胞计数及其在CD4+淋巴细胞的比例以及Th1/Th2比例在生存者及非生存者之间未见差异。同样，在生存者与非生存者，未见上述指标随时间的变化。

结论：下调的T淋巴细胞计数包括Th1、Th2及Th17细胞亚群及失衡的Th1/Th2比例发生在ROSC后早期阶段，这可能参与了OHCA患者ROSC后的免疫功能失调。