Overexpression of programmed cell death-1 (PD-1) affects circulatory Th1 and Th2 cells in patients with cardiac arrest in the early period after the return of spontaneous circulation

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To the Editor: Cardiac arrest (CA) is the leading cause of morbidity and mortality among hospitalized patients globally and has low rates of overall survival. After the return of spontaneous circulation (ROSC), patients with CA and systemic ischemia/reperfusion injury can manifest as sepsis-like syndromes and immune disorders that increase the risk of infections. CD4$^+$ T lymphocytes play an important role in organ ischemia-reperfusion injury. T-helper (Th) types 1 and 2 cells are classical subsets of CD4$^+$ T cells. Extensive research on their functions in infectious and immune-related inflammatory diseases indicates that both subsets participate in host defense and direct immune responses. Data from CA/cardiopulmonary resuscitation porcine models and patients have revealed imbalances in Th1/Th2 cell ratio.[1] Therefore, Th1 and Th2 cells are important for understanding post-ROSC immune dysfunction in patients with CA.

The inhibitory receptor programmed cell death-1 (PD-1) regulates the homeostasis of T cell activation, tolerance, and immunopathology. PD-1 is expressed on activated T cells, natural killer cells, and B cells. PD-1 inhibits interferon (IFN)-γ production and Th1-mediated cellular responses while enhancing IL-4- and Th2-mediated responses. Upregulated PD-1 expression on regulatory T (Treg) cells may be related to immune regulatory disorders in patients with CA.[2] Additionally, immune deficiency in patients with sepsis is associated with enhanced PD-1 or programmed cell death ligand-1 expression. However, whether PD-1 expression on Th1 and Th2 cells is correlated with post-ROSC immune dysfunction remains unknown.

The retrospective study was approved by the Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University (No. 2013-KE-1), and conformed to the *Declaration of Helsinki*. The requirement for informed consent was waived. Biomarker expression was quantified in residual blood after the completion of routine tests every morning. We performed enrolment assessments of patients with CA after their admission to the emergency departments between October 2018 and September 2019. The inclusion criteria were patients with CA > 6 and <24 h after ROSC and Glasgow coma score <8. Patients were excluded if they were <18 years old and had markedly infected organs or tissues, at the end stage of any malignancy or acquired immunodeficiency syndrome, and administered immunosuppressants within the last 3 months. The control group was recruited after a physical examination and comprised healthy age- and gender-matched individuals during the same period.

For enrolled patients, we collected demographic information, laboratory parameters, Acute Physiology and Chronic Health Evaluation II, and Sequential Organ Failure Assessment scores. Residual blood samples from routine clinical tests or physical health examinations were collected and maintained at 4°C during transport and storage before being examined. Supplementary Figure 1, http://links.lww.com/CM9/A757 depicts the workflow of the study. Antibody staining was performed after peripheral blood samples were stimulated for 5 h. The Gallios^{TM} flow cytometer (Beckman Coulter, Brea, CA, USA) and accompanying software (version 1.0) were used for analysis (for antibodies and reagents, see Supplementary Table 1, http://links.lww.com/CM9/A757). Flow-count fluorescent spheres (Beckman Coulter) were used to determine absolute counts of CD3$^+$ lymphocytes. CD4$^+$ T lymphocytes were represented by the CD3$^+$CD8$^-$ population. Th1 and Th2 cells were represented by the CD3$^+$CD8$^-$IFN-γ$^+$ and CD3$^+$CD8$^-$IL-4$^+$ populations, respectively. The flow cytometry gating strategy is shown in Supplementary

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Figure 2, http://links.lww.com/CM9/A757. Plasma cytokines interleukin-6 (IL-6) and IL-10 were quantified using a ProcartaPlex Assay System (Thermo Fisher Scientific, Waltham, MA, USA). Assays were read on the Luminex 200 Multiplexing Instrument (Bio-Rad, Berkeley, CA, USA). For normally distributed data, continuous variables were presented as means and standard deviations. For data with skewed distributions, variables were expressed as median (25th and 75th percentiles). The Mann-Whitney U test was performed for two group comparisons. Qualitative parameters were analyzed using 2×2 contingency table followed by the Chi-square test or Chi-squared test with continuity correction.

The study included 92 patients with CA and 40 healthy individuals (for demographic and clinical characteristics, see Supplementary Table 2, http://links.lww.com/CM9/A757; for patient characteristics based on 28-day survival, see Supplementary Table 3, http://links.lww.com/CM9/A757). Immediately following ROSC, patients with CA had significantly lower Th1 (5.72 [0.97, 25.94] vs. 41.69 [21.75, 87.30] cells/μL, $Z = -5.353$, $P < 0.001$) and Th2 (0.73 [0.26, 1.86] vs. 0.97 [0.65, 2.33] cells/μL, $Z = -2.142$, $P = 0.032$) cell counts, as well as Th1/Th2 cell ratios (7.79 [3.58, 18.30] vs. 34.72 [15.06, 67.67], $Z = -5.674$, $P < 0.001$) than healthy controls. Survivors and non-survivors did not differ significantly in Th1 and Th2 cell counts, Th1/CD4$^+$ and Th2/CD4$^+$ T lymphocytes ratios, or Th1/Th2 lymphocyte ratios ($P > 0.05$) [Supplementary Tables 4 and 5, http://links.lww.com/CM9/A757]. Patients with CA showed significantly higher percentages of PD-1$^+$ Th1 (29.95 [20.88, 41.90]% vs. 24.00 [18.30, 29.73]%, $Z = -2.696$, $P = 0.007$) and Th2 (29.30 [19.63, 39.60]% vs. 24.65 [17.75, 29.10]%, $Z = -2.659$, $P = 0.008$) cells after ROSC as compared with healthy controls [Figures 1A and 1B], but survivors and non-survivors did not differ significantly (30.00 [21.90, 42.45]%
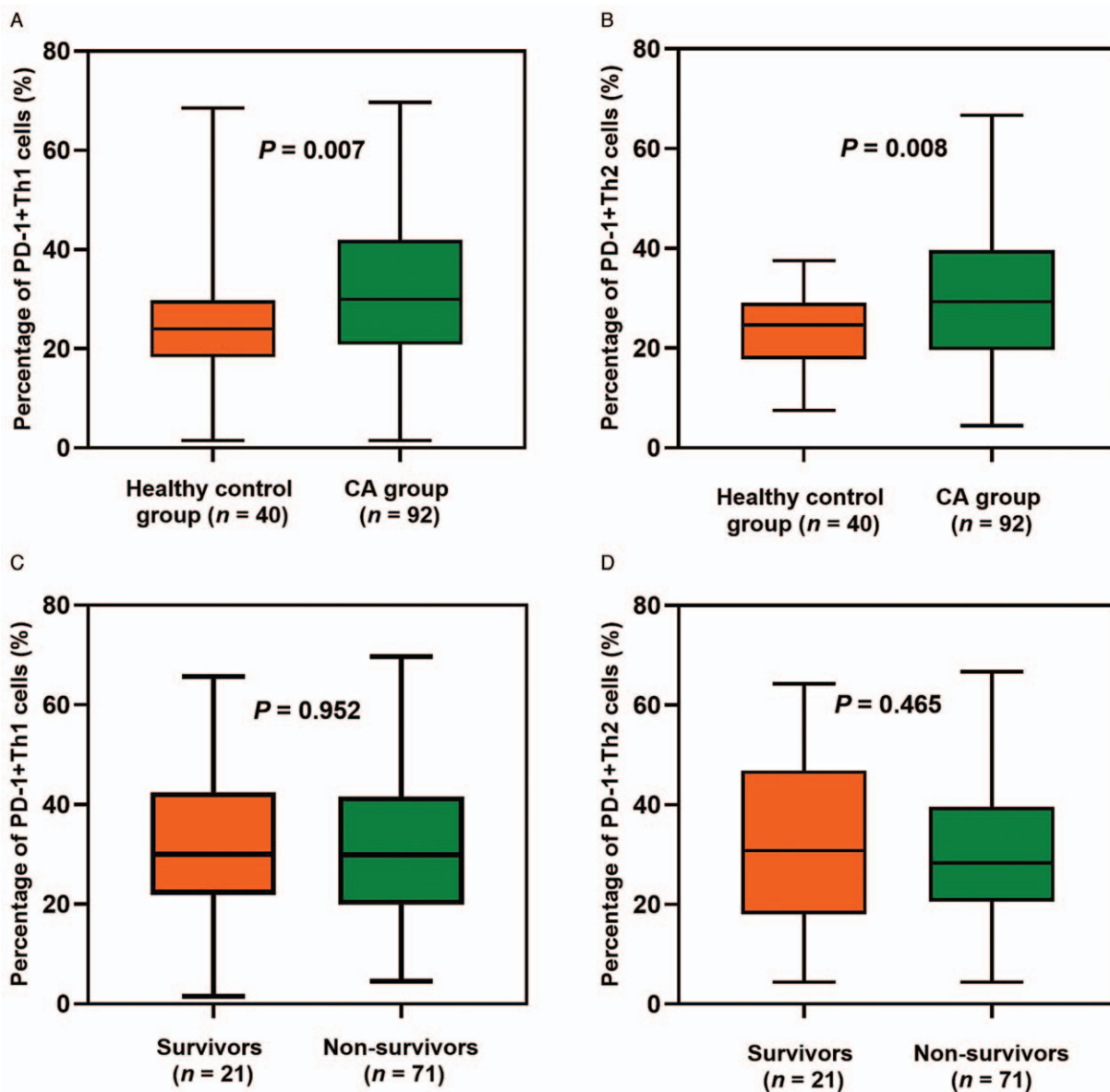

Figure 1: PD-1 expression on circulatory Th1 (A) and Th2 (B) cells in patients with CA after ROSC vs. healthy controls and that (C and D) in survivors vs. non-survivors. CA: Cardiac arrest; PD-1: Programmed cell death-1; ROSC: Return of spontaneous circulation; Th: T-helper.

vs. 29.90 [19.90, 41.60]%, $Z = -0.060$, $P = 0.956$ and 30.80 [18.10, 46.85]% *vs.* 28.30 [20.60, 39.60]%, $Z = -0.730$, $P = 0.465$) [Figure 1C and 1D].

For IL-6 and IL-10, data were non-normally distributed; hence, we compared the values of ln (IL-6 + 1) and ln (IL-10 + 1) and found that both values were higher in patients with CA after ROSC than those in healthy individuals (1.54 [−0.41, 4.16] *vs.* 0.24 [−4.61, 2.42], $Z = -2.346$, $P = 0.019$; 3.95 [−2.30, 5.26] *vs.* −2.30 [−2.30, 4.60], $Z = -2.149$, $P = 0.032$, respectively) [Supplementary Figure 3A and 3B, http://links.lww.com/CM9/A757]. We did not observe significant differences between survivors and non-survivors (0.77 [−4.61, 3.58] *vs.* 1.57 [0.21, 4.33], $Z = -1.363$, $P = 0.173$; 2.00 [−2.30, 4.57] *vs.* 4.26 [−2.30, 5.66], $Z = -1.834$; $P = 0.067$, respectively) [Supplementary Figure 3C and 3D, http://links.lww.com/CM9/A757].

After ROSC following CA, systemic ischemia/reperfusion, with associated burst of reactive oxygen species, causes generalized activation of immunologic pathways, thus increasing the risk of multiple organ failure and infection, similar to sepsis.[3] In clinical conditions, patients with CA are prone to post-ROSC infection. During an adaptive immune response, Th lymphocytes are the main effector cells and have a protective role against pathogenic microorganisms. Patients with sepsis experience pathologic changes, such as an inverse proportion of Th1/Th2 cells, indicating the occurrence of considerable immunosuppression in septic shock. In our study, patients with CA had low Th1 and Th2 cell counts and Th1/Th2 cell ratios after ROSC, consistent with previous research.[1] Additionally, patients with CA experienced an elevation in percentage of PD-1+ Th1 and PD-1+ Th2 cells immediately following ROSC. Similarly, previous studies identified a post-ROSC relative increase in PD-1+ Treg cell percentage among patients with CA, and T lymphocytes in patients with severe sepsis also had enhanced PD-1 expression.[2]

PD-1 is seen as a central regulator of T cell exhaustion. A systematic review and meta-analysis revealed that PD-1 blockade can improve the survival of animals with sepsis.[4] *In vitro* PD-1 blockade decreases apoptosis and improves immune function of lymphocytes from septic patients.[5] Other *in vitro* research shows that PD-1 inhibits Th1 responses by inducing Treg cells and stimulating Th2 responses. Therefore, enhanced PD-1 expression may be a major reason for the Th1/Th2 balance to shift in favor of Th2 and probably also explains immunosuppression in patients with CA after ROSC, increasing their susceptibility to infection. In this study, PD-1 expression on Th cells is similar between survivor and non-survivor. Patients were likely in a state of stress at 6 h after ROSC, leading to only a small difference between the survival and non-survival groups. However, this difference may become significant over time. At present, meager research is available regarding how PD-1 expression on Th cells is linked to pathophysiological mechanisms of CA and sepsis. We suggest that quantification of PD-1 expression levels on Th cells is potentially a way to evaluate early immune dysfunction in patients with CA after ROSC.

We observed a significant increase in plasma pro-inflammatory cytokine IL-6 and anti-inflammatory cytokine IL-10 immediately following ROSC in patients with CA, consistent with the results of previous studies. IL-6 induces Th2 cell differentiation, and IL-10 regulates excessive Th1 cell responses, indicating that high plasma levels of both cytokines during the early post-ROSC period are associated with Th1/Th2 imbalance. Recent studies of the tumor microenvironment showed that IL-6 and PD-1 jointly participate in immune suppression.[6] Patients with high PD-1 expression had elevated IL-10 levels during septic shock.[7] Additionally, the severity of post-CA syndrome is associated with IL-6 and IL-10 release, whereas high IL-6 levels are independently associated with increased mortality in patients with CA.[8] These results collectively suggest that immune dysregulation prevents effective control of inflammation, causing excessive inflammatory cytokine release and increasing the risk of nosocomial infection and multi-organ dysfunction syndrome.

In conclusion, patients with CA exhibited enhanced PD-1 expression, imbalanced Th1/Th2 ratios, and increased plasma IL-6 and IL-10 levels immediately following ROSC. Immune regulation disorders in these patients may be related to Th1 and Th2 cells with upregulated PD-1 expression.

Conflicts of interest

None.

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