Expression of Glucocorticoid Receptors in the Early Period After the Return of Spontaneous Circulation Among Patients Who Experienced Cardiac Arrest : A Retrospective Observational Study

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Abstract

Background: Rapid changes in glucocorticoid (GC) levels and adrenal insufficiency are related to the development of post-cardiac arrest syndrome. However, changes in glucocorticoid receptors (GR) have not been studied. Hence, this study aimed to investigate the association of early changes in GR and prognosis and immune response in patients who experienced cardiac arrest (CA).

Methods: In this observational single-center case-control study, we enrolled patients who were in the early period of return of spontaneous circulation after CA and were admitted to the emergency department of the Beijing Chaoyang Hospital between October 2018 and October 2019. Age- and sex-matched healthy individuals were recruited for the control group after a physical examination.

GR expression and cell counts of circulatory T and B lymphocytes, natural killer, and regulatory T (Treg) cells were assessed. Plasma total cortisol and adrenocorticotrophic hormone (ACTH) levels were tested. Since the data for total cortisol and ACTH levels had a skewed distribution, we compared our results with the natural logarithmic conversion values after adding 1 (ln [total cortisol+ 1], ln [ACTH+ 1]). Measurement data with a skewed distribution are expressed as medians (25th and 75th percentiles). The Mann–Whitney U test was used to compare variables between groups. The qualitative parameters in the 2 × 2 contingency table were used for analysis.

Results: Overall, 85 patients who experienced CA and 40 healthy individuals were enrolled. All cell counts were lower and plasma total cortisol levels were higher ($P<0.001$) in patients who experienced CA than those in the healthy control group. GR expression in Treg cells and $CD3^{+}CD4^{+}$ T lymphocytes was not significantly different, but the mean fluorescence intensity and GR expression in other cells were lower in patients who experienced CA ($P<0.05$) than those in the healthy control group. ACTH levels did not show any difference. There were no significant differences between survivors and non-survivors.

Conclusion: Our findings provide insights into GC sensitivity and immunosuppressive status in these patients, and a new perspective for GC targeted treatment.

Background

Cardiac arrest (CA) is an important health problem globally; about 356,500 people experience medical emergencies due to CA in the United States, and over 544,000 people die from sudden CA in China annually [1–3]. The systemic ischemia-reperfusion response in patients who experienced CA can present as post-cardiac arrest syndrome (PCAS), systematic inflammatory response syndrome (SIRS), which increases the risk of multiple organ failure and infection and affect the inflammatory response and the prognosis of patients [4–7] after return of spontaneous circulation (ROSC).

CA is the most intense among acute stress events, which seriously affects the function of the pituitary and adrenal axis [8]. Studies have shown that abnormal cortisol levels and relative adrenocortical insufficiency after ROSC in patients who experienced CA are related to their prognosis [8–12]. However,

the clinical application of glucocorticoids (GCs) is controversial. In the 2015 International Cardiopulmonary Resuscitation Guidelines, the routine use of GCs is not recommended for resuscitation of patients with in-hospital or out-of-hospital cardiac arrest [13]. Recent clinical studies have shown that early administration of corticosteroids after CA can improve the success rate of ROSC, nervous system functional outcome, and prognosis, which is speculated to be related to its influence on hemodynamics, SIRS response, and other mechanisms [14–18]. Therefore, the role of glucocorticoids in the occurrence and development of PCAS needs to be studied further.

GCs combine with intracellular glucocorticoid receptors (GRs) to exert anti-inflammatory and immunosuppressive effects, and reduce the production as well as the release of inflammatory cytokines [19, 20]. The affinity of GRs to GC in circulating monocytes is decreased in patients with acquired immunodeficiency syndrome [21]. The expression of GR is decreased in critical illness [22], pediatric septic shock, and patients with high serum cortisol [23]. However, hitherto, no study has reported on the GR expression after ROSC in patients who experienced CA. Previous studies have found that the count of circulating B and T lymphocytes, regulatory T (Treg) cells, monocytes, and the human leukocyte antigen DR (HLA-DR) expression on circulatory monocytes and B and T lymphocytes were reduced [24, 25]. Hence, this study aimed to investigate the relationship between GR expression and immune alteration in the early period after ROSC in patients who experienced CA by observing the GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells, their cell counts, and plasma total cortisol and ACTH levels.

Methods

Study participants

This was an observational single-center case-control study conducted in the Emergency Department (ED) of Beijing Chaoyang Hospital in China.

This study was approved by the Medical Ethics Committee of Beijing Chaoyang Hospital.
Due to the observational, non-interventional nature of the study, which was based on residual blood samples taken every morning after measuring the levels of routine biomarkers, we did not obtain informed consent from the patients.
Following the 2015 International Cardiopulmonary Resuscitation Guidelines [26], we enrolled patients who were in the early period of ROSC after CA, and were admitted to the ED of Beijing Chaoyang Hospital between October 2018 and October 2019. The inclusion criteria were (a) ROSC 6 h after CA and (b) a Glasgow coma scale score of < 8 after ROSC. The exclusion criteria were (a) < 18 years of age, (b) terminal stage of disease (malignant cancer of any type, acquired immunodeficiency syndrome), (c) corticosteroid treatment within the past 3 months, (d) administration of corticosteroids, and (e) adrenal insufficiency. All patients were treated according to the 2015 International Cardiopulmonary Resuscitation Consensus [13]. Age- and sex-matched healthy individuals were recruited for the control group after a physical examination.

Data collection

We collected data on demographics, resuscitation (initial heart rhythm, ROSC time, and cumulative adrenaline [epinephrine] dose), as well as laboratory findings (routine blood cell, blood gas, and blood biochemical tests performed 6 h after ROSC). Acute Physiology and Chronic Health Evaluation (APACHE) II and the Sequential Organ Failure Assessment (SOFA) were used to determine disease severity. Residual samples of blood, with heparin anticoagulant, from routine clinical tests or physical health examinations were collected, maintained at 4°C during transport and storage, and used to determine GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells and their cell counts. The plasma was maintained at -80°C during storage and used to determine total cortisol and ACTH levels. During follow-up, 28-day survival data were also collected. Additional file 1, shows the workflow of this study.

Flow cytometry

Expression of GR was measured on T and B lymphocytes, NK cells, and Treg cells. Briefly, a 100-μL peripheral blood sample was stained for 20 min with surface antibodies (CD3, CD4, CD8, CD19, CD16, CD56, CD25, and CD127) in a dark place. Erythrocytes were lysed for 15 min, and the debris was washed away. Before intracellular GR staining, surface-stained cells were fixed and permeabilized using the BD Transcription Factor Buffer Set (BD Pharmingen™, Catalogue No. 562574). Monoclonal antibodies and their isotype controls were all purchased from BD Biosciences (San Jose, CA, USA). Details of all antibodies are shown in additional file 2. According to the manufacturers' recommendations, all antibodies and their isotype controls were used in the concentration of 1 μL per 100 μL whole blood. Samples were measured using the Gallios flow cytometer (Beckman Coulter, Brea, CA) and analyzed on the Gallios Software version 1.0 (Beckman Coulter). Cells were stained for 20 min; thresholds were defined using the manufacturer's recommended isotype controls. T cells were gated by $CD3^+$ $CD4^+$ or $CD3^+$ $CD8^+$; B cells were gated by $CD3^-$ $CD19^+$; NK cells were gated by $CD16^+$ $CD56^+$; Tregs were gated by $CD4^+CD25^{high}CD127^{low}$. At least 10,000 events were collected in the lymphocyte cell gate for each sample. Results are expressed as percentages and mean fluorescence intensity (MFI) values.

Absolute $CD3^+$ and $CD4^+$ lymphocytes, NK cell, and Treg cell counts were obtained using Flow-Count fluorospheres (Beckman Coulter, Catalogue No. 7547053), according to the manufacturer's instructions. B, $CD3^+CD4^+$T, $CD3^+CD8^+$T, and Treg cell counts were calculated by their percentages in $CD3^+$ or $CD4^+$ lymphocytes multiplied by $CD3^+$ or $CD4^+$ lymphocyte counts.

Determination of plasma total cortisol and ACTH levels after ROSC

Venous blood samples were collected in ethylenediaminetetraacetic acid tubes, centrifuged, and then stored at -80°C. Plasma total cortisol (IMMULITE2000 Cortisol, L2KCO2, United Kingdom) and ACTH (IMMULITE2000 ACTH, L2KAC2, United Kingdom) were assayed using a chemiluminescent immunoassay on a Siemens automated analyzer (Immulite® 2000 XPi; Siemens Healthcare Diagnostics,

Erlangen, Germany). The lower detection limit of total cortisol was 2.00 ng/mL, and that of ACTH was 5.00 pg/mL.

Statistical analyses

All data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 8.0 (GraphPad Software). For normally distributed data, continuous variables are expressed as means with standard deviations. Since the data for total cortisol and ACTH levels had a skewed distribution, we compared our results with the natural logarithmic conversion values after adding 1 (ln [total cortisol + 1], ln [ACTH + 1]). Measurement data with a skewed distribution are expressed as medians (25th and 75th percentiles). The Mann–Whitney U test was used to compare variables between groups. The qualitative parameters in the 2 × 2 contingency table were used for analysis. All statistical tests were two-tailed, and a P-value of < 0.05 was considered statistically significant.

Follow-up

Patients who experienced CA were classified into survivor and non-survivor groups according to the 28-day survival end-point. Those with all-cause mortality within the follow-up period were considered as non-survivors.

Results

Patient characteristics

In total, 40 healthy individuals and 85 patients who experienced CA were analyzed. The demographics and clinical characteristics of both groups are shown in Table 1. In this study, acute cardiac and brain events were the main causes of CA. Other causes of CA included poisoning (including carbon monoxide poisoning) and hypokalemia. Sex and age were not significantly different between the CA and healthy control groups. The comparisons of clinical characteristics of the survivor and non-survivor groups based on 28-day survival are shown in additional file 3. The APACHE II and SOFA scores were significantly different between the CA and healthy control groups ($P < 0.001$ for all) and the survivor and non-survivor groups ($P < 0.001$ and $P = 0.011$, respectively).

Table 1
Patient Characteristics at Admission

Characteristics	Healthy Control Group (n = 40)	Successful Resuscitation Group (n = 85)	P-Value
Age (years), median [IQR]	64.0 (54.3–69.8)	65.0 (55.0–74.0)	0.209
Male/Female (n)	23/17	58/27	0.241
Previous medical history, n (%)			
Hypertension	5 (12.5%)	38 (44.7%)	< 0.001
Diabetes	3 (7.5%)	27 (31.8%)	0.003
Coronary heart disease	2 (5.0%)	29 (34.1%)	< 0.001
Chronic lung disease	1 (2.5%)	9 (10.6%)	0.230
Chronic kidney disease	0	9 (10.6%)	0.077
Cardiac arrest cause (n, %)			
Cardiac		34 (40.0%)	
Respiratory		20 (23.5%)	
Cerebral		23 (27.1%)	
Others		7 (8.2%)	
Unknow		1 (1.2%)	
Initial resuscitation			
Time to ROSC (min), median [IQR]		20.0 (10.0–30.0)	
Adrenaline (mg), median [IQR]		2.0 (0.0–5.0)	
Initial rhythm VF/VT, n (%)		30 (35.3%)	
MAP (mmHg), median [IQR]95.7 (86.0-103.2)		74.3 (56.2–97.2)	< 0.001
White cell count ($\times 10^9$/L), median [IQR]	5.81 (4.85–6.53)	13.56 (10.84–18.29)	< 0.001

Data are presented as mean ± SD or interquartile range (IQR) as appropriate. The p-value represents comparison between groups. Abbreviations: ROSC: return of spontaneous circulation; VF: ventricular fibrillation; VT: ventricular tachycardia; MAP: mean arterial pressure; APACHE Ⅱ: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; CPC: cerebral performance category.

Characteristics	Healthy Control Group (n = 40)	Successful Resuscitation Group (n = 85)	P-Value
APACHE score, mean ± SD	0	32.9 ± 6.5	< 0.001
SOFA score, median [IQR]	0	11.5 (8.5–14.0)	< 0.001
28-day mortality, n (%)		65(76.5%)	
28-day CPC 1–2, n (%)		14 (16.5%)	

Data are presented as mean ± SD or interquartile range (IQR) as appropriate. The p-value represents comparison between groups. Abbreviations: ROSC: return of spontaneous circulation; VF: ventricular fibrillation; VT: ventricular tachycardia; MAP: mean arterial pressure; APACHE : acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; CPC: cerebral performance category.

Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts after ROSC

Compared with those of the healthy controls, the T and B lymphocyte, NK cell, and Treg cell counts were significantly lower after ROSC in patients who experienced CA ($P < 0.001$ for all). Compared with those of the healthy controls, the $CD3^+CD4^+$/T lymphocyte, $CD3^+CD8^+$/T lymphocyte, and Treg cell/$CD4^+$ T lymphocyte ratios were also significantly reduced after ROSC in patients who experienced CA ($P < 0.001$ for all) (Fig. 1; Additional file 4). However, there were no significant differences in these cell counts and ratios between survivors (n = 20) and non-survivors (n = 65) ($P > 0.05$ for all) (Additional file 5).

GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells after ROSC

Compared with those in healthy individuals, the MFI and percentages of GR expression in B and T lymphocytes, NK cells, and $CD3^+CD8^+$ T lymphocytes decreased significantly after ROSC in patients who experienced CA ($P < 0.01$ for all) (Figs. 2A–D, G, H, K, L). There were also significant reductions in the MFI of GR expression in Treg cells and $CD3^+CD4^+$ T lymphocytes ($P < 0.05$ for all) (Figs. 2E, I) but none in the percentages of GR expression ($P > 0.05$ for all) (Figs. 2F, J; Additional file 6). However, there were no significant differences in the MFI and percentages of GR expression in these cells between survivors and non-survivors ($P > 0.05$ for all) (Additional file 7).

Changes in plasma total cortisol and ACTH levels after ROSC

We measured the plasma total cortisol and ACTH levels of the 40 healthy individuals and 85 CA patients (two samples were excluded because their total cortisol levels were not measured). Compared with those in healthy controls, plasma total cortisol levels were significantly higher in patients who experienced CA ($P < 0.001$) but ACTH levels were not (Figs. 3A, C). No statistical differences in ln (total cortisol + 1) and ln (ACTH + 1) values were observed between survivors and non-survivors ($P > 0.05$ for all) (Figs. 3B, D).

Discussion

This study explored the relationship between GR expression and immune alteration in the early period after ROSC in patients who experienced CA, by observing GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells and changes in cell counts and plasma total cortisol and ACTH levels. We found that GR expression, cell counts, and ratios rapidly decreased and plasma total cortisol levels increased in these patients.

After ROSC, the immune response of patients who experienced CA is impaired, and the systemic inflammatory response is increased [7, 27]. In this study, the T and B lymphocyte, NK cell, and Treg cell counts as well as the $CD3^+CD4^+$/T, $CD3^+CD8^+$/T, and Treg cell/$CD4^+$T lymphocyte ratios were significantly reduced after ROSC. NK cells, which are special innate immune cells that have cytotoxic functions similar to $CD3^+CD8^+$ T lymphocytes, mainly distinguish infected and stressed cells from healthy cells and eliminate intracellular infection as well as dysfunctional cells [28, 29]. T lymphocytes are also important because of their function as adaptive immune cells for the control and elimination of infection [28]. Moreover, B and T lymphocytes mediate humoral and cellular immunity, respectively. Compared with previous studies, this study further performed an earlier and more comprehensive assessment of the immune system of patients who experienced CA, and our findings more substantially supported the rapid emergence of immune dysfunction in these patients after ROSC compared to that of previous reports.

The effectiveness of GC use in these patients during and after resuscitation has been controversial due to insufficient evidence. However, the use of GC during resuscitation improves the survival rate of patients who experienced CA due to its direct anti-inflammatory, immunosuppressive effects, hemodynamic, and positive inotropic effects. All of this ultimately leads to increased stress capacity of the body [19, 20]. GC can activate GRs in cells when the body is under stress, thereby increasing both the effectiveness of resuscitation and the discharge survival rate. This study is the first to explore GR expression in circulating immune cells in patients who experienced CA after ROSC. We observed that GR expression in B and T lymphocytes, NK cells, and $CD3^+CD8^+$ T lymphocytes decreased significantly in patients who experienced CA, while the percentage of GR^+ Treg cells and $CD3^+CD4^+$ T lymphocytes showed a slight decrease. Moreover, we observed a more significant decrease in the MFI of GR expression in Treg cells and $CD3^+CD4^+$ T lymphocytes but none in the percentage of GR expression. Previous studies have found decreased expression of GRs in peripheral polymorphonuclear cells in critically ill patients [22], and antagonism to GRs aggravates viral and bacterial infections [30]. The results of this study suggest that

the decrease in intracellular GR expression in patients who experienced CA may be one of the causes of GC resistance, due to insufficient binding of GRs and GCs, GC insensitivity, and the inability of GCs to effectively exert anti-inflammatory and immunosuppressive effects. These findings may also explain why different results of the clinical application of GCs have appeared in previous studies and support the possibility of using GCs in the clinical treatment of patients who experienced CA.

In this study, we also found that the total plasma cortisol levels were significantly higher in patients who experienced CA but ACTH levels were not. High levels of inflammatory cytokines inhibit ACTH release [19]. During critical illness, the body does not sufficiently metabolize cortisol [31]. In addition, the continuous increase in plasma cortisol levels may trigger the negative feedback pathway of the hypothalamic-pituitary-adrenal axis, inhibiting the release of ACTH and cortisol and eventually leading to adrenal insufficiency. These factors may explain the opposite trends of plasma ACTH and cortisol levels in the patients who were included in this study and experienced CA. Notably, this result suggests that low GR expression levels cannot be matched with high plasma total cortisol levels. Previous studies have found that GC use during resuscitation may benefit patients who experienced CA [14–17]. The benefits, such as direct anti-inflammatory and anti-shock effects, improvement of vascular endothelial permeability, and other mechanisms may be related to the effects of using a high dose of GC, or GCs may work through other non-GR pathways. It is also possible that the immune function of patients who experienced CA is suppressed due to ischemia-reperfusion injury, which requires a large dose of GC to stimulate GRs to function. This study did not provide data on plasma GC levels and GR expression in a group of patients who were administered GCs and successfully resuscitated; therefore, further studies are required to explore the exact mechanisms of GCs.

Our study has several limitations. First, to assess obvious changes, we only enrolled patients who experienced CA and had obvious signs of systemic ischemic hypoxia, such as GCS < 8 after ROSC. The patients were not stratified by age, gender, occurrence of comorbidities or mild systemic ischemic hypoxia. Second, since this was a preliminary observational study, we were only observing early changes. A dynamic observation for a longer duration would be helpful to understand the significance of GR expression in evolving immunity during the clinical course of CA after ROSC. Third, the samples used in this study were from the clinical laboratory, so the plasma total cortisol and ACTH in the samples were at a risk of degradation before we collected the samples. Finally, we did not discuss the changes in and the roles of GR isoforms, free cortisol, and corticosteroid-binding globulin. Therefore, future studies on these aspects are warranted to better understand the immunosuppressive effects of ROSC among patients who experienced CA.

Conclusion

In conclusion, this study revealed that GR expression, cell counts, and ratios rapidly decreased, while plasma total cortisol levels increased in the early period after ROSC among patients who experienced CA. These findings may provide important information about GC sensitivity and immunosuppressive status

in these patients. In addition, this study provides a new perspective for clinical targeted treatment using GCs and high-quality prognosis in patients who experienced CA.

Abbreviations

GC: Glucocorticoids; GR: Glucocorticoid receptors; CA: Cardiac arrest; Treg: Regulatory T cells; ACTH: Adrenocorticotrophic hormone; PCAS: Post cardiac arrest syndrome; SIRS: Systematic inflammatory response syndrome; ROCS: Return of spontaneous circulation; HLA-DR: Human leukocyte antigen DR; ED: Emergency department; APACHE: Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Assessment; MFI: Mean fluorescence intensity; NK: Natural killer cells

Declarations

Ethics approval: This study was approved by the Medical Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University (2013-KE-1).

Consent to participate: Due to the observational, non-interventional nature of the study, which was based on residual blood samples taken every morning after measuring the levels of routine biomarkers, we did not obtain informed consent from the patients.

Consent to publish: Not applicable**Availability of data and materials:** All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests: The authors declare that they have no competing interests.

Funding: None

Author contributions: CL designed the study and reviewed the manuscript. YNY searched literatures, contributed to experimental studies, date analysis and writing this manuscript. ZRT, CCH and LA collected and analyzed data. JBL and MRX helped with statistical analysis. All authors have read and approved the final manuscript.

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Tables

Table 1. Patient Characteristics at Admission

Characteristics	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)	P-Value
Age (years), median [IQR]	64.0 (54.3-69.8)	65.0 (55.0-74.0)	0.209
Male/Female (n)	23/17	58/27	0.241
Previous medical history, n (%)			
Hypertension	5 (12.5%)	38 (44.7%)	<0.001
Diabetes	3 (7.5%)	27 (31.8%)	0.003
Coronary heart disease	2 (5.0%)	29 (34.1%)	<0.001
Chronic lung disease	1 (2.5%)	9 (10.6%)	0.230
Chronic kidney disease	0	9 (10.6%)	0.077
Cardiac arrest cause (n, %)			
Cardiac		34 (40.0%)	
Respiratory		20 (23.5%)	
Cerebral		23 (27.1%)	
Others		7 (8.2%)	
Unknow		1 (1.2%)	
Initial resuscitation			
Time to ROSC (min), median [IQR]		20.0 (10.0-30.0)	
Adrenaline (mg), median [IQR]		2.0 (0.0-5.0)	
Initial rhythm VF/VT, n (%)		30 (35.3%)	
MAP (mmHg), median [IQR] (86.0-103.2)	95.7	74.3 (56.2-97.2)	<0.001
White cell count (×10^9/L), median [IQR]	5.81 (4.85-6.53)	13.56 (10.84-18.29)	<0.001
APACHE score, mean±SD	0	32.9±6.5	<0.001
SOFA score, median [IQR]	0	11.5 (8.5-14.0)	<0.001
28-day mortality, n (%)		65 76.5%	
28-day CPC 1–2, n (%)		14 (16.5%)	

Data are presented as mean±SD or interquartile range (IQR) as appropriate. The p-value represents comparison between groups. Abbreviations: ROSC: return of spontaneous circulation; VF: ventricular

fibrillation; VT: ventricular tachycardia; MAP: mean arterial pressure; APACHE Ⅱ: acute physiology and chronic health evaluation; SOFA: sequential organ failure assessment; CPC: cerebral performance category.

Figures

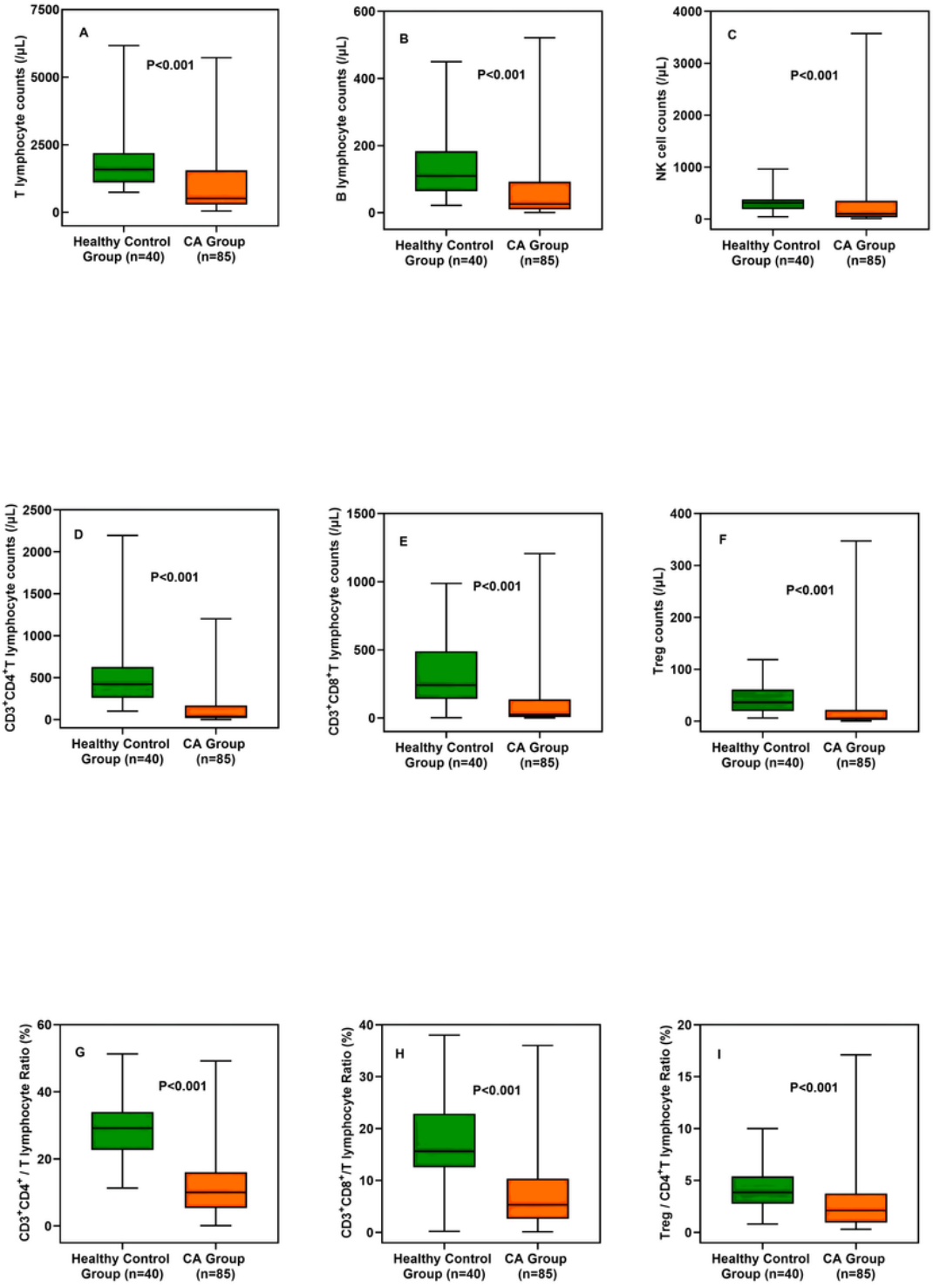

Figure 1

Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and CD3+CD4+/T, CD3+CD8+/T, and Treg/CD4+T lymphocyte ratios between the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group (P<0.001). CA, cardiac arrest; NK, natural killer; Treg, regulatory T.

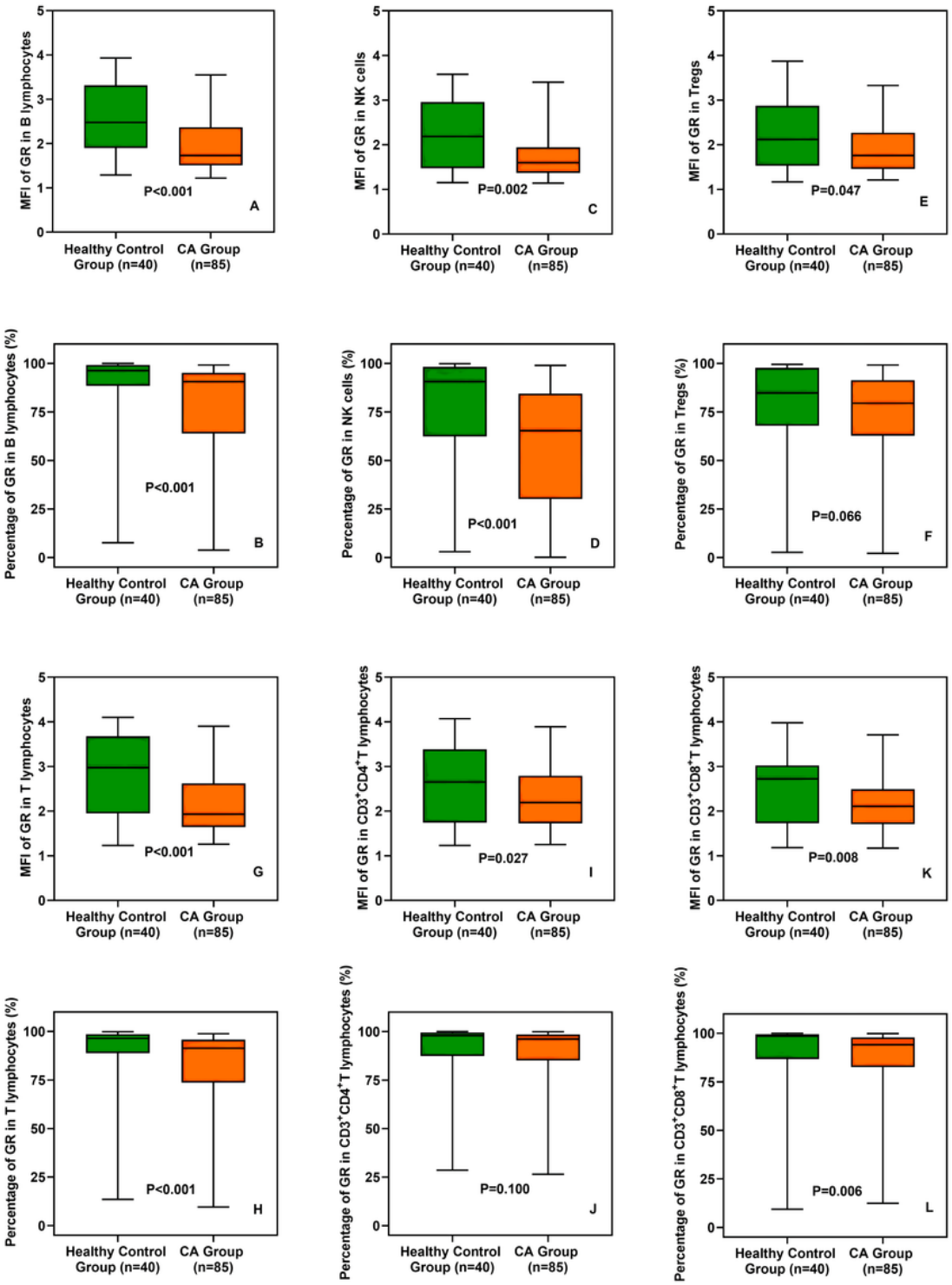

Figure 2

Expression of GRs in circulatory T and B lymphocytes, NK cells, and Treg cells in the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group (P<0.05). CA, cardiac arrest; GR, glucocorticoid receptor; NK, natural killer; ROSC, return of spontaneous circulation; Treg, regulatory T.

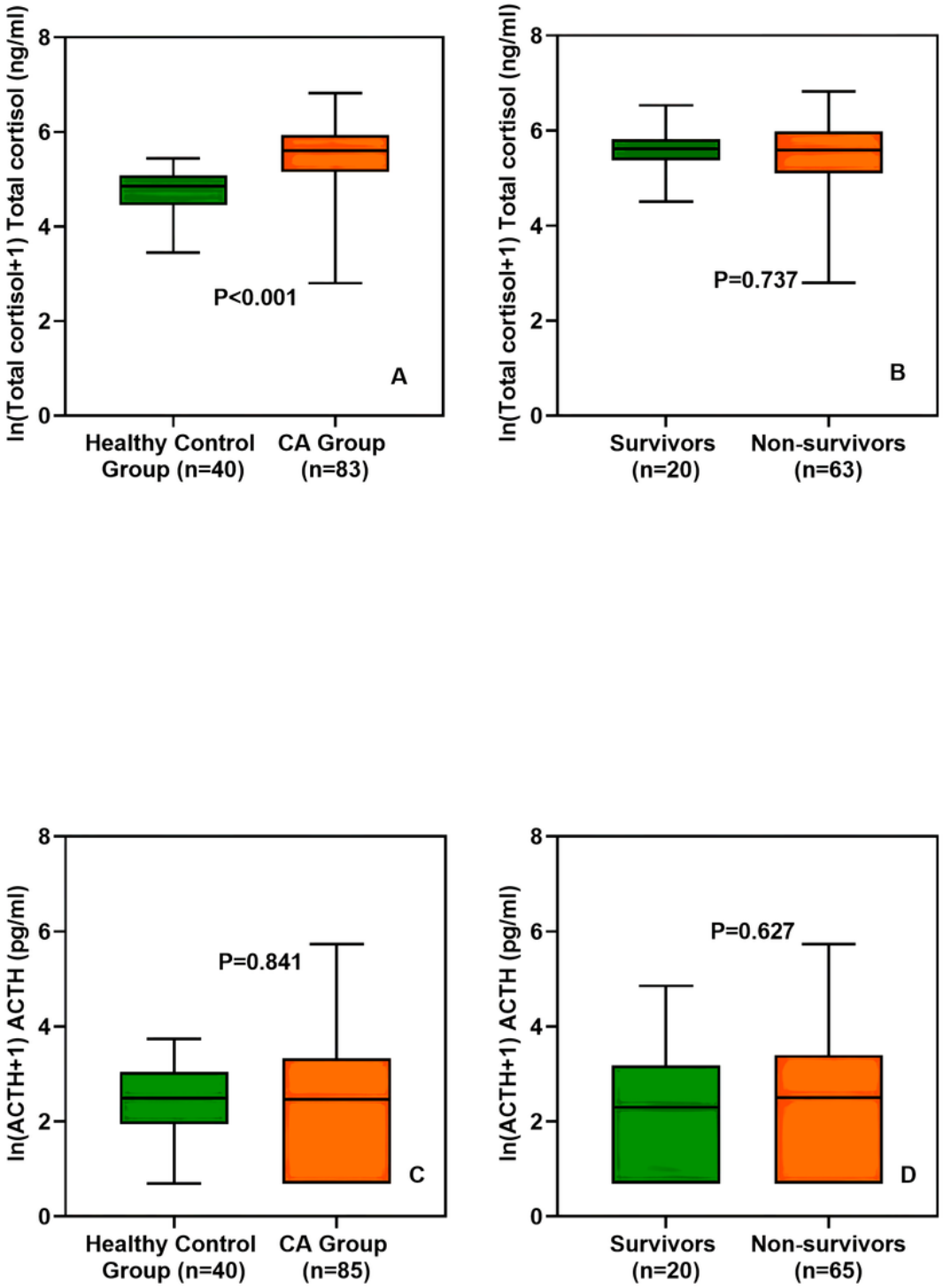

Figure 3

(A, B) The (ln+1) values of plasma total cortisol and ACTH levels after ROSC in the healthy control group and CA group. (C, D) The (ln+1) values of plasma total cortisol and ACTH levels in survivors and non-survivors after ROSC. The CA group showed significant differences compared with the healthy control group ($P<0.05$). ACTH, adrenocorticotrophic hormone; CA, cardiac arrest; ROSC, return of spontaneous circulation.

Supplementary Files

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- Electronicsupplementalmaterial.docx