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Glucocorticoid receptor expression after the return of spontaneous circulation in patients who experienced cardiac arrest: A prospective observational study

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1 **Glucocorticoid receptor expression after the return of spontaneous circulation in**
2 **patients who experienced cardiac arrest: A prospective observational study**

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15 **Word count:** 3199

23 Abstract

24 **Objectives:** Rapid changes in glucocorticoid (GC) levels and adrenal insufficiency are
25 related to the development of post-cardiac arrest (CA) syndrome. However, changes in
26 GC receptor (GR) expression have not been studied. Hence, the aim of this study was
27 to investigate the association of early changes in GR expression and prognosis and
28 immune response in patients who experienced CA.

29 **Design:** Prospective observational study.

30 **Setting:** Emergency department.

31 **Participants:** Patients (85) who were in the early period of return of spontaneous
32 circulation (ROSC) after CA and were admitted between October 2018 and October
33 2019. Age- and sex-matched healthy individuals (40) were recruited for the control
34 group after a physical examination.

35 **Primary and secondary outcome measures:** GR expression and cell counts of
36 circulatory T and B lymphocytes, natural killer cells, and regulatory T (Treg) cells were
37 assessed. Plasma total cortisol and adrenocorticotrophic hormone (ACTH) levels were
38 also tested.

39 **Results:** All cell counts were lower, and plasma total cortisol levels were higher
40 ($P<0.001$), in patients who experienced CA than in the healthy control group. GR
41 expression in Treg cells and $CD3^+CD4^+$ T lymphocytes was not significantly different,
42 but the mean fluorescence intensity and GR expression in other cells were lower in
43 patients who experienced CA ($P<0.05$) than in the healthy control group. ACTH levels
44 were not different. There were no significant differences between survivors and non-

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4 45 survivors.

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6 46 **Conclusions:** This study revealed that GR expression and cell counts rapidly decreased,
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9 47 whereas plasma total cortisol levels increased, in the early period after ROSC among
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12 48 patients who experienced CA. Our findings provide insights into GC sensitivity and
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15 49 immunosuppressive status in these patients, and a new perspective for GC targeted
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17 50 treatment.

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20 51 **Strengths and limitations of this study**

- 21
22 52 1. Explore whether controversy over glucocorticoid use is associated with different
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25 53 levels of glucocorticoid receptor expression in cardiac arrest patients for the first
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28 54 time.
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30 55 2. Glucocorticoid receptor expression rapidly decreased in the early period following
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33 56 restoration of spontaneous circulation among patients who experienced cardiac arrest .
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35 57 3. We only observed changes in glucocorticoid receptor expression of cardiac
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38 58 arrest patients at the early period following restoration of spontaneous circulation, and
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41 59 long-term dynamic observation would be helpful to understand the significance of
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44 60 clinical steroid therapy.

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46 61 **Introduction**

47
48 62 Cardiac arrest (CA) is an important health problem globally; about 356,500 people
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51 63 experience medical emergencies due to CA in the United States, and over 544,000
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54 64 people die from sudden CA in China annually. [1, 2] The systemic ischemia-reperfusion
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57 65 response in patients who have experienced CA can present as post-CA syndrome
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60 66 (PCAS) or systematic inflammatory response syndrome (SIRS), which increases the

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4 67 risk of multiple organ failure and infection and affects the inflammatory response and
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6 68 prognosis of patients after the return of spontaneous circulation (ROSC). [3-6]
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9 69 CA is the most intense among acute stress events, which seriously affect the function
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11 70 of the pituitary and adrenal axis. [7] Studies have shown that abnormal cortisol levels
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13 71 and relative adrenocortical insufficiency after ROSC in patients who experienced CA
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15 72 are related to their prognosis. [8-11] However, the clinical application of
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17 73 glucocorticoids (GCs) is controversial. In the 2015 International Cardiopulmonary
18
19 74 Resuscitation Guidelines, the routine use of GCs is not recommended for the
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21 75 resuscitation of patients with in-hospital or out-of-hospital CA. [12] Recent clinical
22
23 76 studies have shown that early administration of corticosteroids after CA can improve
24
25 77 the success rate of ROSC, nervous system functional outcome, and prognosis, which is
26
27 78 speculated to be related to its influence on hemodynamics, SIRS response, and other
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29 79 mechanisms. [12-17] Therefore, the role of GCs in the occurrence and development of
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31 80 PCAS needs to be studied further.
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40 81 GCs combine with intracellular GC receptors (GRs) to exert anti-inflammatory and
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42 82 immunosuppressive effects and reduce the production as well as release of
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44 83 inflammatory cytokines. [18, 19] The affinity of GRs to GCs in circulating monocytes
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46 84 is decreased in patients with acquired immunodeficiency syndrome. [20] The
47
48 85 expression of GR is decreased in patients with critical illness, [21] pediatric septic
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50 86 shock, and high serum cortisol level. [22] However, hitherto, no study has reported the
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52 87 GR expression after ROSC in patients who experienced CA. Previous studies have
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54 88 found that the counts of circulating B and T lymphocytes, regulatory T (Treg) cells, and
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4 89 monocytes and expression of human leukocyte antigen DR (HLA-DR) on circulatory
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6 90 monocytes and B and T lymphocytes are reduced. [23, 24] Hence, the aim of this study
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9 91 was to investigate the relationship between GR expression and immune alteration in the
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11 92 early period after ROSC in patients who experienced CA by observing GR expression
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14 93 in circulatory T and B lymphocytes, NK cells, and Treg cells, their cell counts, and
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16
17 94 plasma total cortisol and adrenocorticotrophic hormone (ACTH) levels.
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21 22 96 **MATERIALS AND METHODS**

23 24 25 97 **Study participants**

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27 98 This was an observational study conducted in the Emergency Department (ED).
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30 99 Following the 2015 International Cardiopulmonary Resuscitation Guidelines, [25] we
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32 100 enrolled patients who were in the early period of ROSC after CA and were admitted to
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34
35 101 the ED between October 2018 and October 2019. The inclusion criteria were (a) ROSC
36
37 102 6 h after CA and (b) Glasgow Coma Scale score <8 after ROSC. The exclusion criteria
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39
40 103 were (a) <18 years of age, (b) terminal stage of disease (such as cancer of any type,
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42
43 104 acquired immunodeficiency syndrome), (c) corticosteroid treatment within the past 3
44
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46 105 months, (d) administration of corticosteroids, and (e) adrenal insufficiency. All patients
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49 106 were treated according to the 2015 International Cardiopulmonary Resuscitation
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51 107 Consensus. [13] Age- and sex-matched healthy individuals were recruited for the
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53
54 108 control group after a physical examination.
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57 58 110 **Data collection**

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4 111 We collected data on demographics, resuscitation (initial heart rhythm, ROSC time,
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6 112 and cumulative adrenaline epinephrine dose), and laboratory findings (routine blood
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9 113 cell counts, blood gas analysis, and blood biochemical tests performed 6 h after ROSC).
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11 114 Acute Physiology and Chronic Health Evaluation (APACHE) II and the Sequential
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14 115 Organ Failure Assessment (SOFA) were used to determine disease severity. Residual
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17 116 samples of blood, with heparin anticoagulant, from routine clinical tests or physical
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20 117 health examinations were collected, maintained at 4 °C during transport and storage,
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22
23 118 and used to determine GR expression in circulatory T and B lymphocytes, NK cells,
24
25 119 and Treg cells and their cell counts. The plasma was maintained at -80 °C during storage
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27
28 120 and used to determine total cortisol and ACTH levels. During follow-up, 28-day
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30 121 survival data were also collected. Supplemental Figure 1 shows the workflow of this
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33 122 study.

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37 124 **Flow cytometry**

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40 125 GR expression in T and B lymphocytes, NK cells, and Treg cells was measured. Briefly,
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43 126 a 100- μ L peripheral blood sample was stained for 20 min with surface antibodies (CD3,
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46 127 CD4, CD8, CD19, CD16, CD56, CD25, and CD127) in a dark place. Erythrocytes were
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49 128 lysed for 15 min, and the debris was washed away. Before intracellular GR staining,
50
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52 129 surface-stained cells were fixed and permeabilized using the BD Transcription Factor
53
54
55 130 Buffer Set (BD Pharmingen, San Diego, USA, Catalogue No. 562574). Monoclonal
56
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58 131 antibodies and their isotype controls were all purchased from BD Biosciences (San
59
60 132 Jose, CA, USA). Details of all antibodies are shown in Supplemental Table 1.

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4 133 According to the manufacturer's recommendations, all antibodies and their isotype
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6 134 controls were used at a concentration of 1 μL per 100 μL of whole blood. Samples were
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9 135 measured using the Gallios flow cytometer (Beckman Coulter, Brea, CA, USA) and
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11 136 analyzed using Gallios Software version 1.0 (Beckman Coulter). The flow cytometer
12
13
14 137 was periodically calibrated by an engineer. Cells were stained for 20 min; thresholds
15
16
17 138 were defined using the manufacturer's recommended isotype controls. T cells were
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19 139 gated by $\text{CD3}^+\text{CD4}^+$ or $\text{CD3}^+\text{CD8}^+$, B cells were gated by $\text{CD3}^-\text{CD19}^+$, NK cells were
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22 140 gated by $\text{CD16}^+\text{CD56}^+$, and Tregs were gated by $\text{CD4}^+\text{CD25}^{\text{high}}\text{CD127}^{\text{low}}$. At least
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24
25 141 10,000 events were collected in the lymphocyte cell gate for each sample. Results are
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27
28 142 expressed as percentages and mean fluorescence intensity (MFI) values.

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30 143 Absolute CD3^+ and CD4^+ lymphocyte, NK cell, and Treg cell counts were obtained
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32 144 using Flow-Count fluorospheres (Beckman Coulter, Catalogue No. 7547053),
33
34
35 145 according to the manufacturer's instructions. B, $\text{CD3}^+\text{CD4}^+\text{T}$, $\text{CD3}^+\text{CD8}^+\text{T}$, and Treg
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37
38 146 cell counts were calculated by their percentages in CD3^+ or CD4^+ lymphocytes
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41 147 multiplied by CD3^+ or CD4^+ lymphocyte counts.

42 43 44 45 149 **Determination of plasma total cortisol and ACTH levels after ROSC**

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48 150 Venous blood samples were collected in ethylenediaminetetraacetic acid tubes,
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51 151 centrifuged 10 min at 3000 rpm, and then stored at $-80\text{ }^\circ\text{C}$. Plasma total cortisol
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54 152 (IMMULITE 2000 Cortisol, L2KCO2, UK) and ACTH (IMMULITE 2000 ACTH,
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57 153 L2KAC2, UK) levels were assayed using a chemiluminescent immunoassay on a
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60 154 Siemens automated analyzer (IMMULITE 2000 XPi; Siemens Healthcare Diagnostics,

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4 155 Erlangen, Germany). The equipment and reagents were calibrated by engineers before
5
6 156 use. The lower detection limit of total cortisol was 2.00 ng/mL and that of ACTH was
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9 157 5.00 pg/mL.
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13 14 159 **Statistical analyses**

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17 160 All data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). For
18
19 161 normally distributed data, continuous variables are expressed as means with standard
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22 162 deviations. Since the data for total cortisol and ACTH levels had a skewed distribution,
23
24 163 we compared our results with the natural logarithmic conversion values after adding 1
25
26
27 164 (\ln [total cortisol+ 1], \ln [ACTH+ 1]). Measurement data with a skewed distribution are
28
29
30 165 expressed as medians (25th and 75th percentiles). The Mann–Whitney U test was used
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33 166 to compare variables between groups. The qualitative parameters in the 2×2
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35 167 contingency table were used for analysis. All statistical tests were two-tailed, and a P-
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38 168 value of <0.05 was considered statistically significant.
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42 43 170 **Follow-up**

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45 171 Patients who experienced CA were classified into survivor and non-survivor groups
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48 172 according to the 28-day survival endpoint. Those with all-cause mortality within the
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51 173 follow-up period were considered non-survivors. If data were lost, the corresponding
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54 174 candidate was excluded.
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58 59 176 **Patient and public involvement**

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4 177 This study was approved by the Medical Ethics Committee (2013-KE-1). Patient
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6 178 consent to participate was obtained prior to enrolment in this study.
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10 11 180 **Results**

12 13 181 **Patient characteristics**

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17 182 In total, 40 healthy individuals and 85 patients who experienced CA were analyzed.
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19 183 The demographics and clinical characteristics of both groups are shown in Table 1. In
20
21 184 this study, acute cardiac and brain events were the main causes of CA. Other causes of
22
23 185 CA included poisoning (including carbon monoxide poisoning) and hypokalemia. Sex
24
25 186 and age were not significantly different between the CA and healthy control groups.
26
27 187 The comparisons of clinical characteristics of the survivor and non-survivor groups
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29 188 based on 28-day survival are shown in Supplemental Table 2. The APACHE II and
30
31 189 SOFA scores were significantly different between the CA and healthy control groups
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33 190 ($P < 0.001$ for all) and survivor and non-survivor groups ($P < 0.001$ and $P = 0.011$,
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35 191 respectively).
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46 193 **Table 1.** Patient Characteristics at Admission

Characteristics	Healthy Control	Successful	P-value
	Group (n=40)	Resuscitation Group (n=85)	
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		20.0 (10.0, 30.0)	
[IQR]			
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,	74.3 (56.2, 97.2)	<0.001
	103.2)		
White cell count ($\times 10^9/L$), median	5.81 (4.85, 6.53)	13.56 (10.84, 18.29)	<0.001
[IQR]			
APACHE II score, mean \pm SD	0	32.9 \pm 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%)	

194 Abbreviations: IQR: interquartile range; ROSC: return of spontaneous circulation;
 195 VF: ventricular fibrillation; VT: ventricular tachycardia; MAP: mean arterial pressure;
 196 APACHE II: acute physiology and chronic health evaluation; SOFA: sequential
 197 organ failure assessment; SD: standard deviation; CPC: cerebral performance
 198 category.

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6 **200 Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts after**
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8 **201 ROSC**

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11 202 The T and B lymphocyte, NK cell, and Treg cell counts were significantly lower after
12
13 203 ROSC in patients who experienced CA than in healthy controls ($P < 0.001$ for all).
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15 204 Additionally, the $CD3^+CD4^+/T$ lymphocyte, $CD3^+CD8^+/T$ lymphocyte, and Treg
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17 205 cell/ $CD4^+$ T lymphocyte ratios were significantly lower after ROSC in patients who
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19 206 experienced CA than in healthy controls ($P < 0.001$ for all) (Fig. 1; Supplemental Table
20
21 207 3). However, there were no significant differences in these cell counts and ratios
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23 208 between survivors ($n=20$) and non-survivors ($n=65$) ($P > 0.05$ for all) (Supplemental
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25 209 Table 4).
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35 **211 GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells after**
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37 **212 ROSC**

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40 213 The MFI and percentages of GR expression in B and T lymphocytes, NK cells, and
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42 214 $CD3^+CD8^+$ T lymphocytes were significantly lower after ROSC in patients who
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44 215 experienced CA than in healthy individuals ($P < 0.01$ for all) (Fig. 2A–D, G, H, K, L).
45
46 216 There were also significant reductions in the MFI in Treg cells and $CD3^+CD4^+$ T
47
48 217 lymphocytes ($P < 0.05$ for all) (Figs. 2E, I) but not in the percentages of GR expression
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50 218 ($P > 0.05$ for all) (Figs. 2F, J; Supplemental Table 5). However, there were no significant
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52 219 differences in the MFI and percentages of GR expression in these cells between
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54 220 survivors and non-survivors ($P > 0.05$ for all) (Supplemental Table 6).
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222 **Changes in plasma total cortisol and ACTH levels after ROSC**

223 We measured the plasma total cortisol and ACTH levels of the 40 healthy individuals
224 and 85 patients who experienced CA (two samples were excluded because their total
225 cortisol levels were not measured). Plasma total cortisol levels were significantly higher
226 in patients who experienced CA than in healthy controls ($P < 0.001$) but ACTH levels
227 were not (Figs. 3A, C). No significant differences in $\ln(\text{total cortisol}+1)$ and \ln
228 $(\text{ACTH}+1)$ values were observed between survivors and non-survivors ($P > 0.05$ for all)
229 (Fig. 3B, D).

230

231 **Discussion**

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

238 After ROSC, the immune response of patients who experience CA is impaired, and
239 the systemic inflammatory response is increased. [6, 26] In this study, the T and B
240 lymphocyte, NK cell, and Treg cell counts as well as $\text{CD3}^+\text{CD4}^+/\text{T}$, $\text{CD3}^+\text{CD8}^+/\text{T}$, and
241 Treg cell/ CD4^+ T lymphocyte ratios were significantly reduced after ROSC. NK cells,
242 which are special innate immune cells that have cytotoxic functions similar to

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4 243 CD3⁺CD8⁺ T lymphocytes, mainly distinguish infected and stressed cells from healthy
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6 244 cells and eliminate intracellular infection as well as dysfunctional cells. [27, 28] T
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9 245 lymphocytes are also important because of their function as adaptive immune cells for
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11 246 the control and elimination of infection. [27] Moreover, B and T lymphocytes mediate
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14 247 humoral and cellular immunity, respectively. This study was performed at an earlier
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17 248 period and involved a more comprehensive assessment of the immune system of
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19 249 patients who experienced CA, and our findings more substantially supported the rapid
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22 250 emergence of immune dysfunction in these patients after ROSC than previous reports.
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26 251 The effectiveness of GC use in these patients during and after resuscitation has been
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28 252 controversial due to insufficient evidence. However, the use of GCs during resuscitation
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31 253 improves the survival rate of patients who experience CA due to its direct anti-
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33 254 inflammatory, immunosuppressive effects, hemodynamics, and positive inotropic
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36 255 effects. All of this ultimately leads to an increased stress capacity of the body. [18, 19]
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39 256 GCs can activate GRs in cells when the body is under stress, thereby increasing both
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41 257 the effectiveness of resuscitation and discharge survival rate. This study is the first to
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44 258 explore GR expression in circulating immune cells in patients who experienced CA
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46 259 after ROSC. We observed that GR expression in B and T lymphocytes, NK cells, and
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49 260 CD3⁺CD8⁺ T lymphocytes decreased significantly in patients who experienced CA,
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51 261 whereas the percentage of GR⁺ Treg cells and CD3⁺CD4⁺ T lymphocytes showed a
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54 262 slight decrease. Moreover, we observed a more significant decrease in the MFI of GR
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57 263 expression in Treg cells and CD3⁺CD4⁺ T lymphocytes but not in the percentage of GR
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4 264 expression. Previous studies have found decreased expression of GRs in peripheral
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6 265 polymorphonuclear cells in critically ill patients, [21] and antagonism to GRs
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9 266 aggravates viral and bacterial infections. [29] The results of this study suggest that the
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11 267 decrease in intracellular GR expression in patients who experienced CA is one of the
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14 268 causes of GC resistance, due to insufficient binding of GRs and GCs, GC insensitivity,
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17 269 and the inability of GCs to effectively exert anti-inflammatory and immunosuppressive
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20 270 effects. These findings may also explain why different results regarding the clinical
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22 271 application of GCs have been reported previously and support the possibility of using
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25 272 GCs in the clinical treatment of patients who experienced CA.

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27 273 We also found that the total plasma cortisol levels were significantly higher in
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30 274 patients who experienced CA, but ACTH levels were not. High levels of inflammatory
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33 275 cytokines inhibit ACTH release. [18] During critical illness, the body does not
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36 276 sufficiently metabolize cortisol. [30] In addition, the continuous increase in plasma
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39 277 cortisol levels may trigger the negative feedback pathway of the hypothalamic-
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42 278 pituitary-adrenal axis, inhibiting the release of ACTH and cortisol and eventually
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45 279 leading to adrenal insufficiency. These factors may explain the opposite trends of
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48 280 plasma ACTH and cortisol levels in the patients who were included in this study and
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50
51 281 experienced CA. Notably, this result suggests that low GR expression levels are not
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54 282 matched with high plasma total cortisol levels. Previous studies have found that GC use
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57 283 during resuscitation may benefit patients who experience CA. [13-16] The benefits,
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60 284 such as direct anti-inflammatory and anti-shock effects, improvement of vascular
285 endothelial permeability, and other mechanisms may be related to the effects of using

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4 286 a high dose of GCs, or GCs may work through other non-GR pathways. It is also
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6 287 possible that the immune function of patients who experience CA is suppressed due to
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9 288 ischemia-reperfusion injury, which requires a large dose of GCs to stimulate GRs to
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12 289 function. This study did not provide data on plasma GC levels and GR expression in a
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14 290 group of patients who were administered GCs and successfully resuscitated; therefore,
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16
17 291 further studies are required to explore the exact mechanisms of GCs.

292 **Limitations**

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

305 In conclusion, this study revealed that GR expression, cell counts, and ratios
306 rapidly decreased, whereas plasma total cortisol levels increased, in the early period
307 after ROSC among CA patients. These findings may provide important information

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4 308 about GC sensitivity and immunosuppressive status in these patients. In addition, this
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6 309 study provides a new perspective for clinical targeted treatment using GCs and high-
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9 310 quality prognosis in CA patients.
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27 317 **Contributorship statement:** CL designed the study and reviewed the manuscript.
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30 318 YNY searched the literature and contributed to the experimental studies, data
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33 319 analysis, and writing of the manuscript. ZRT, CCH, and LA collected and analyzed
34
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36 320 data. JBL and MRX helped with the statistical analyses. All authors have read and
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38 321 approved the final manuscript.

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56 328 not be shared.

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For peer review only

332 References

- 333 1 Myat A, Song KJ, Rea T. Out-of-hospital cardiac arrest: current concepts. *Lancet*
334 2018;**391**:970–9.
- 335 2 Zhang S. Sudden cardiac death in China: current status and future perspectives.
336 *Europace* 2015;**17** Supplement 2:ii14–8.
- 337 3 Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology,
338 pathophysiology, treatment, and prognostication. A Scientific Statement from the
339 international Liaison Committee on Resuscitation; the American Heart Association
340 Emergency cardiovascular Care Committee; the Council on Cardiovascular Surgery
341 and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the
342 Council on Clinical Cardiology; the Council on Stroke. *Resuscitation* 2008;**79**:350–79.
- 343 4 Su CP, Wu JH, Yang MC, et al. Demographics and clinical features of
344 postresuscitation comorbidities in long-term survivors of out-of-hospital cardiac arrest:
345 A national follow-up study. *BioMed Res Int* 2017;**2017**:9259182.
- 346 5 Tsai MS, Chiang WC, Lee CC, et al. Infections in the survivors of out-of-hospital
347 cardiac arrest in the first 7 days. *Intensive Care Med* 2005;**31**:621–6.
- 348 6 Adrie C, Adib-Conquy M, Laurent I, et al. Successful cardiopulmonary
349 resuscitation after cardiac arrest as a “sepsis-like” syndrome. *Circulation*
350 2002;**106**:562–8.
- 351 7 Hall ED. Neuroprotective actions of glucocorticoid and nonglucocorticoid steroids
352 in acute neuronal injury. *Cell Mol Neurobiol* 1993;**13**:415–32.
- 353 8 de Jong MF, Beishuizen A, de Jong MJ et al. The pituitary-adrenal axis is activated

-
- 354 more in non-survivors than in survivors of cardiac arrest, irrespective of therapeutic
355 hypothermia. *Resuscitation* 2008;**78**:281–8.
- 356 9 Mosaddegh R, Kianmehr N, Mahshidfar B et al. Serum cortisol level and adrenal
357 reserve as a predictor of patients' outcome after successful cardiopulmonary
358 resuscitation. *J Cardiovasc Thorac Res* 2016;**8**:61–4.
- 359 10 Hékimian G, Baugnon T, Thuong M, et al. Cortisol levels and adrenal reserve after
360 successful cardiac arrest resuscitation. *Shock* 2004;**22**:116–9.
- 361 11 Tavakoli N, Bidari A, Shams Vahdati S. Serum Cortisol Levels as a Predictor of
362 Neurologic Survival in Successfully Resuscitated Victims of Cardiopulmonary Arrest.
363 *J Cardiovasc Thorac Res* 2012;**4**:107–11.
- 364 12 Soar J, Callaway CW, Aibiki M, et al. Resuscitation- Part 4: advanced life support:
365 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency
366 Cardiovascular Care Science with Treatment Recommendations. *Resuscitation*
367 2015;**95**:e71–ee120.
- 368 13 Mentzelopoulos SD, Malachias S, Chamos C, et al. Vasopressin, steroids, and
369 epinephrine and neurologically favorable survival after in-hospital cardiac arrest: a
370 randomized clinical trial. *JAMA* 2013;**310**:270–9.
- 371 14 Tsai MS, Chuang PY, Yu PH, et al. Glucocorticoid use during cardiopulmonary
372 resuscitation may be beneficial for cardiac arrest. *Int J Cardiol* 2016;**222**:629–35.
- 373 15 Niimura T, Zamami Y, Koyama T, et al. Hydrocortisone administration was
374 associated with improved survival in Japanese patients with cardiac arrest [Sci.
375 rep.:17919]. *Sci Rep* 2017;**7**:17919.

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- 1
2
3
4 376 16 Chalkias A, Xanthos T. Post-cardiac arrest syndrome: mechanisms and evaluation
5
6
7 377 of adrenal insufficiency. *World J Crit Care Med* 2012;**1**:4–9.
8
9 378 17 Buddineni JP, Callaway C, Huang DT. Epinephrine, vasopressin and steroids for
10
11 379 in-hospital cardiac arrest: the right cocktail therapy? *Crit Care* 2014;**18**:308.
12
13
14 380 doi:[10.1186/cc13903](https://doi.org/10.1186/cc13903).
15
16
17 381 18 Varvarousi G, Stefanidou A, Varvaroussi D et al. Glucocorticoids as an emerging
18
19 382 pharmacologic agent for cardiopulmonary resuscitation. *Cardiovasc Drugs Ther*
20
21
22 383 2014;**28**:477–88.
23
24
25 384 19 Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease.
26
27 385 *Trends Pharmacol Sci* 2013;**34**:518–30.
28
29
30 386 20 Norbiato G, Bevilacqua M, Vago T, et al. Cortisol resistance in acquired
31
32 387 immunodeficiency syndrome. *J Clin Endocrinol Metab* 1992;**74**:608–13.
33
34
35 388 21 Vassiliou AG, Floros G, Jahaj E, et al. Decreased glucocorticoid receptor
36
37 389 expression during critical illness. *Eur J Clin Invest* 2019;**49**:e13073.
38
39
40 390 22 Alder MN, Opoka AM, Wong HR. The glucocorticoid receptor and cortisol levels
41
42 391 in pediatric septic shock. *Crit Care* 2018;**22**:244.
43
44
45 392 23 Qi Z, Liu Q, Zhang Q et al. Overexpression of programmed cell death-1 and human
46
47 393 leucocyte antigen-DR on circulatory regulatory T cells in out-of-hospital cardiac arrest
48
49 394 patients in the early period after return of spontaneous circulation. *Resuscitation*
50
51 395 2018;**130**:13–20.
52
53
54
55 396 24 Qi Z, An L, Liu B, et al. Patients with out-of-hospital cardiac arrest show decreased
56
57 397 human leucocyte antigen-DR expression on monocytes and B and T lymphocytes after
58
59
60

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- 1
2
3
4 398 return of spontaneous circulation. *Scand J Immunol* 2018;**88**:e12707.
5
6
7 399 25 Perkins GD, Travers AH, Berg RA, et al. Resuscitation-Part 3: adult basic life
8
9 400 support and automated external defibrillation: 2015 International Consensus on
10
11 401 Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with
12
13 402 Treatment Recommendations. *Resuscitation* 2015;**95**:e43–e69.
14
15
16
17 403 26 Beurskens CJ, Horn J, de Boer AM, et al. Cardiac arrest patients have an impaired
18
19 404 immune response, which is not influenced by induced hypothermia. *Crit Care*
20
21 405 2014;**18**:R162.
22
23
24
25 406 27 Lanier LL. NK cell recognition. *Annu Rev Immunol* 2005;**23**:225–74.
26
27 407 28 Vivier E, Tomasello E, Baratin M et al. Functions of natural killer cells. *Nat*
28
29 408 *Immunol* 2008;**9**:503–10.
30
31
32 409 29 Webster JI, Sternberg EM. Role of the hypothalamic-pituitary-adrenal axis,
33
34 410 glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial
35
36 411 and viral products. *J Endocrinol* 2004;**181**:207–21.
37
38
39 412 30 Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during
40
41 413 critical illness. *N Engl J Med* 2013;**368**:1477–88.
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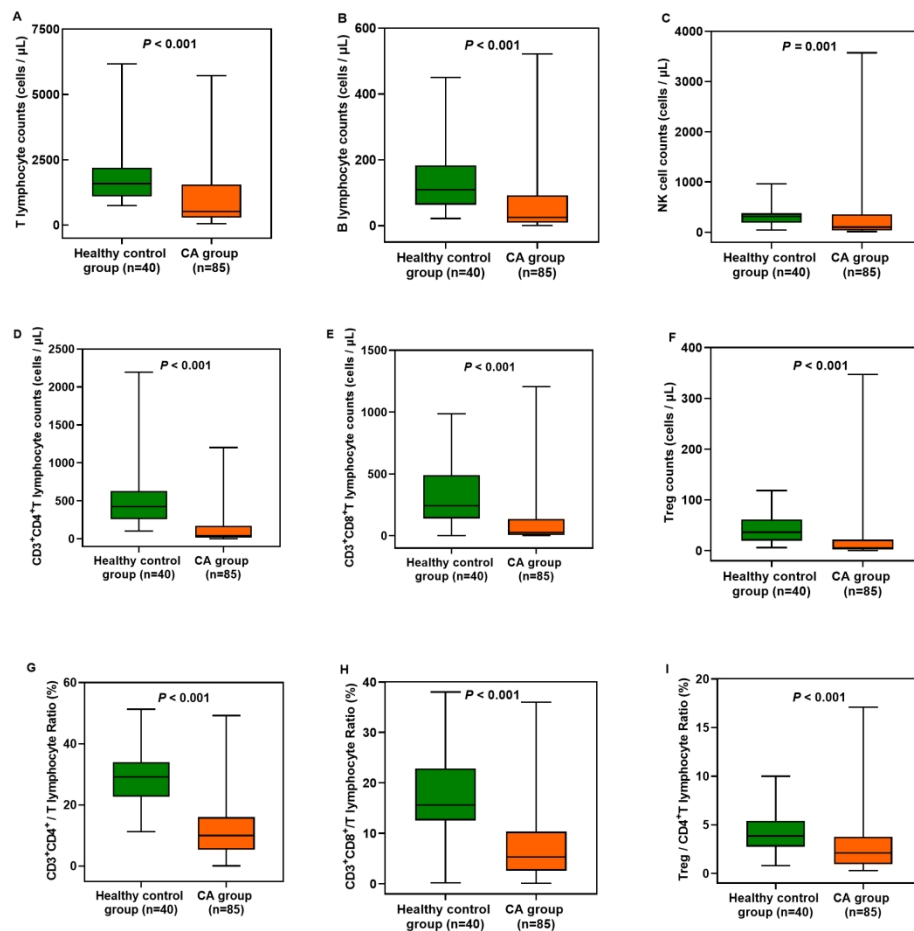
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4 414 **Figure legends**

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6 415 **Fig. 1.** Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and
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9 416 CD3⁺CD4⁺/T, CD3⁺CD8⁺/T, and Treg/CD4⁺T lymphocyte ratios between the healthy
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11 417 control group and CA group. The CA group showed significant differences compared
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14 418 with the healthy control group (P<0.001). CA, cardiac arrest; CD, cluster-of-
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17 419 differentiation; NK, natural killer; Treg, regulatory T.

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19 420 **Fig. 2.** Expression of GRs in circulatory T and B lymphocytes, NK cells, and Treg cells
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22 421 in the healthy control group and CA group. The CA group showed significant
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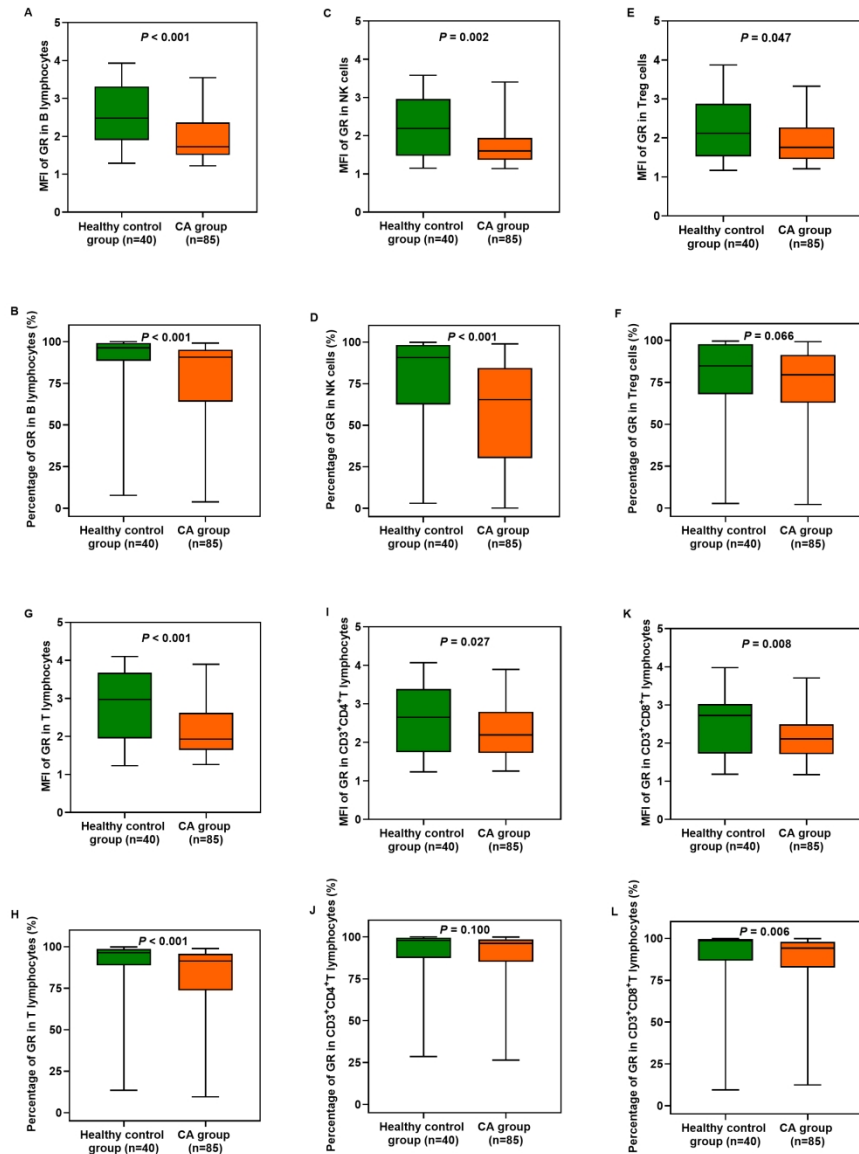
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32 425 **Fig. 3.** (A, B) Plasma total cortisol and ACTH levels (the natural logarithmic
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35 426 conversion values after adding 1) after ROSC in the healthy control group and CA
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38 427 group. (C, D) Plasma total cortisol and ACTH levels in survivors and non-survivors
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41 428 after ROSC. The CA group showed significant differences compared with the healthy
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43 429 control group (P<0.05). ACTH, adrenocorticotrophic hormone; CA, cardiac arrest;
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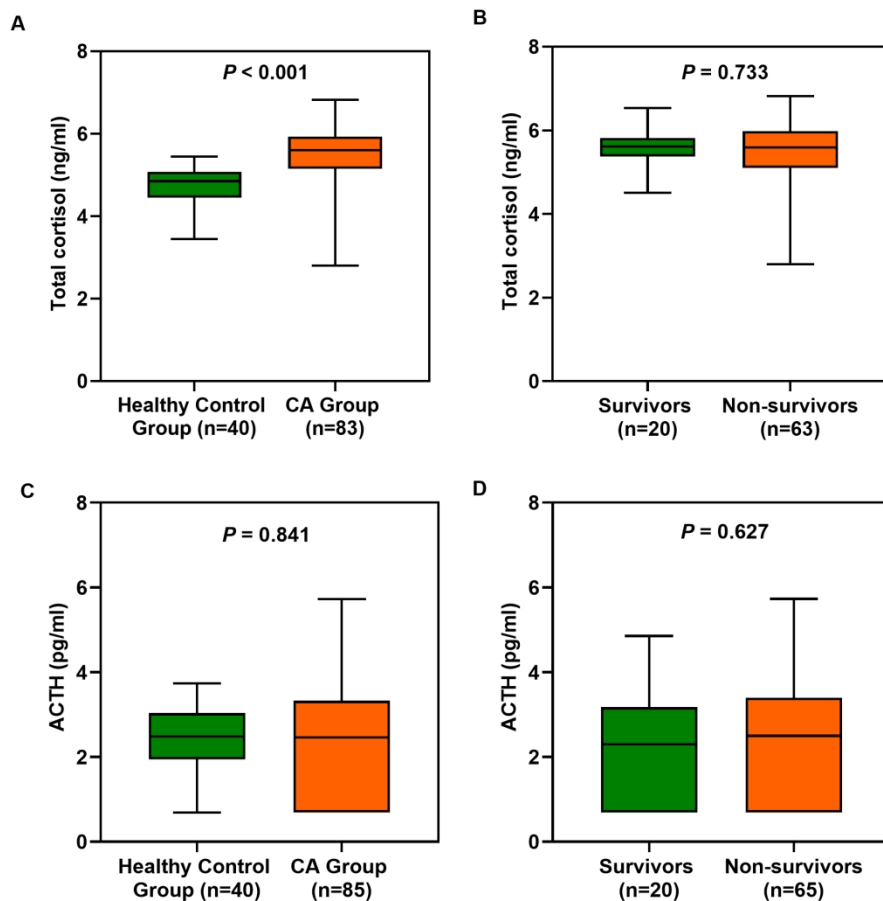
Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and CD3+CD4+/T, CD3+CD8+/T, and Treg/CD4+T lymphocyte ratios between the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group ($P < 0.001$). CA, cardiac arrest; CD, cluster-of-differentiation; NK, natural killer; Treg, regulatory T.

187x183mm (300 x 300 DPI)



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; CD, cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC, return of spontaneous circulation; Treg, regulatory T.

199x256mm (300 x 300 DPI)



(A, B) Plasma total cortisol and ACTH levels (the natural logarithmic conversion values after adding 1) after ROSC in the healthy control group and CA group. (C, D) 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.

185x178mm (300 x 300 DPI)

Electronic supplemental material**Expression of glucocorticoid receptors early after the return of spontaneous circulation in patients who experienced cardiac arrest: A prospective observational study**

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Contents

Supplemental Figure 1

Supplemental Table 1

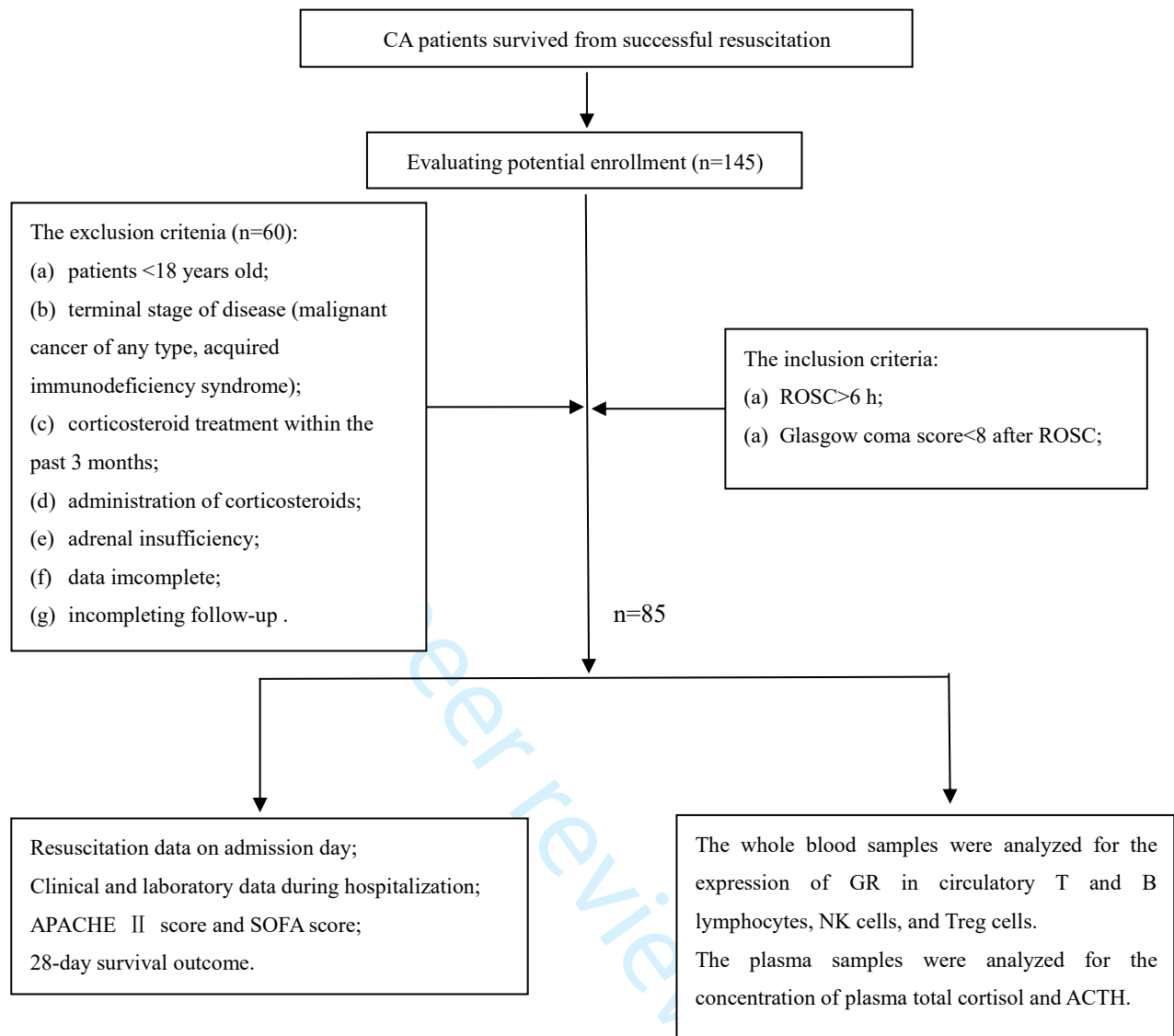
Supplemental Table 2

Supplemental Table 3

Supplemental Table 4

Supplemental Table 5

Supplemental Table 6



Supplemental Figure 1. The flow chart of the study.

Abbreviations: CA, cardiac arrest; ROSC, return of spontaneous circulation; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; GR, glucocorticoid receptor; Treg, regulatory T; ACTH, adrenocorticotrophic hormone.

Supplemental Table 1. Details of antibodies for flow cytometry.

Antigen	Catalog Number	Fluorescein Conjugate	Source
CD3	558117	Pacific Blue	BD Pharmingen ^a
CD4	555347	PE	BD Pharmingen
CD4	560345	Horizon V450	BD Pharmingen
CD8	557746	PE-Cy7	BD Pharmingen
CD19	557835	PE-Cy7	BD Pharmingen
CD16	558122	Pacific Blue	BD Pharmingen
CD56	557747	PE-Cy7	BD Pharmingen
CD25	557741	PE-Cy7	BD Pharmingen
CD127	557938	PE	BD Pharmingen
GR	MCA2469F	FITC	Bio-Rad ^b
Mouse IgG1 Isotype	MCA928F	FITC	Bio-Rad
Mouse IgG1, κ Isotype	557872	PE-Cy7	BD Pharmingen
Mouse IgG1, κ Isotype	554680	PE	BD Pharmingen
Mouse IgG1, κ Isotype	558120	Pacific Blue	BD Pharmingen

^a BD Pharmingen, San Diego, USA; ^b Bio-Rad AbD Serotec, Oxford, UK.

Abbreviations: CD, cluster-of-differentiation; PE, phycoerythrin; FITC, fluorescein isothiocyanate; GR, glucocorticoid receptor; Ig: immunoglobulin.

Supplemental Table 2. Characteristics of CA survivors and non-survivors on admission.

	Survivors (n=20)	Non-survivors (n=65)	P-value
Age (years), median [IQR]	59.0 (53.3, 72.8)	66.0 (59.0, 75.5)	0.070
Male/Female (n)	12/8	46/19	0.366
Cardiac arrest cause (n, %)			
Cardiac	10 (50.0%)	24 (36.9%)	0.297
Non-Cardiac	10 (50.0%)	41 (63.1%)	0.297
Initial resuscitation			
Time to ROSC (min), median [IQR]	15.0 (7.3, 26.0)	20.0 (15.0, 30.0)	0.032
Adrenaline (mg), median [IQR]	1.0 (0.0, 3.0)	2.0 (0.0, 5.0)	0.091
Initial rhythm VF/VT, n (%)	11 (55.0%)	19 (29.2%)	0.035
MAP (mmHg), median [IQR]	89.9 (70.5, 104.9)	70.7 (50.0, 93.5)	0.033
White cell count ($\times 10^9/L$), median [IQR]	12.40 (6.98, 18.76)	13.80 (11.67, 18.20)	0.286
Lactate (mmol/L), median [IQR]	3.50 (1.33, 7.05)	7.50 (3.80, 11.20)	0.008
APACHE II score, mean \pm SD	27.8 \pm 6.6	34.4 \pm 5.6	<0.001
SOFA score, median [IQR]	9.0 (7.3, 11.8)	12.0 (9.0, 15.0)	0.011

Data are presented as mean \pm 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.

Supplemental Table 3. The flow cytometry results of cell counts and ratios of healthy control group and successful resuscitation group

	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)	Z-value	P-value
T lymphocyte count (cells / μ L)	1586.0 (1101.5, 2192.5)	514.0 (287.5, 1555.0)	-4.515	<0.001
NK cell count (/ μ L)	311.5 (191.0, 378.8)	101.0 (36.0, 351.5)	-3.332	0.001
B lymphocyte count (/ μ L)	109.3 (63.7, 183.3)	25.7 (9.4, 92.3)	-5.076	<0.001
Treg count (/ μ L)	0.259 (0.095, 0.516)	0.233 (0.135, 0.488)	-5.518	<0.001
Treg / CD4 ⁺ T lymphocyte Ratio	0.039 (0.028, 0.054)	0.021 (0.010, 0.038)	-4.418	<0.001
CD3 ⁺ CD4 ⁺ T lymphocyte count (/ μ L)	421.7 (258.6, 627.4)	38.9 (17.6, 168.3)	-6.256	<0.001
CD3 ⁺ CD4 ⁺ / T lymphocyte Ratio	0.292 (0.227, 0.340)	0.100 (0.054, 0.160)	-7.066	<0.001
CD3 ⁺ CD8 ⁺ T lymphocyte count (/ μ L)	241.1 (139.5, 488.6)	26.3 (7.2, 135.9)	-5.287	<0.001
CD3 ⁺ CD8 ⁺ / T lymphocyte Ratio	0.157 (0.126, 0.229)	0.053 (0.026, 0.104)	-5.719	<0.001

All the data in Supplemental table 3 are represented as the median [IQR]; IQR: Interquartile Range; CD: cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; Treg, regulatory T.

Supplemental Table 4. The flow cytometry results of cell counts and ratios of the CA patients on admission based on 28-day survival

	Survivors (n=20)	Non-survivors (n=65)	Z-value	P-value
T lymphocyte count (/μL)	502.0 (353.8, 1199.8)	514.0 (282.5, 1891.0)	-0.186	0.852
NK cell count (/μL)	167.0 (29.8, 309.3)	100.0 (36.0, 404.0)	-0.218	0.828
B lymphocyte count (/μL)	38.6 (15.7, 103.5)	19.2 (7.1, 65.7)	-0.632	0.527
Tregs count (/μL)	0.318 (0.145, 0.552)	0.212 (0.128, 0.479)	-0.611	0.396
Treg / CD4 ⁺ T lymphocyte Ratio	0.025 (0.009, 0.043)	0.021 (0.010, 0.034)	-0.498	0.619
CD3 ⁺ CD4 ⁺ T lymphocyte count (/μL)	55.1 (32.4, 228.0)	38.0 (16.0, 168.1)	-0.850	0.396
CD3 ⁺ CD4 ⁺ / T lymphocyte Ratio	0.118 (0.070, 0.236)	0.097 (0.049, 0.142)	-1.565	0.118
CD3 ⁺ CD8 ⁺ T lymphocyte count (/μL)	25.4 (12.5, 96.2)	26.3 (6.3, 138.8)	-0.021	0.983
CD3 ⁺ CD8 ⁺ / T lymphocyte Ratio	0.054 (0.033, 0.104)	0.053 (0.025, 0.104)	-0.187	0.852

All the data in Supplemental table 4 are represented as the median [IQR]; IQR: Interquartile Range; CD: cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; Treg, regulatory T.

Supplemental Table 5. The flow cytometry results of GR expression in the CA group and successful resuscitation group.

	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)	Z-value	P-value
Percentage of GR on B lymphocytes	0.963 (0.885, 0.992)	0.896 (0.605, 0.949)	-3.742	<0.001
MFI of GR on B lymphocytes	2.48 (1.91, 3.31)	1.73 (1.50, 2.37)	-3.980	<0.001
Percentage of GR on T lymphocytes	0.964 (0.889, 0.986)	0.900 (0.703, 0.955)	-3.755	<0.001
MFI of GR on T lymphocytes	2.98(1.95, 3.68)	1.92 (1.36, 1.99)	-3.853	<0.001
Percentage of GR on NK cells	0.907 (0.624, 0.983)	0.611 (0.306, 0.840)	-3.792	<0.001
MFI of GR on NK cells	2.19 (1.48, 2.96)	1.60 (1.36, 1.99)	-3.171	0.002
Percentage of GR on Treg cells	0.848 (0.680, 0.978)	0.784 (0.589, 0.911)	-1.837	0.066
MFI of GR on Treg cells	2.12 (1.53, 2.88)	1.76 (1.44, 2.30)	-1.990	0.047
Percentage of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	0.980 (0.874, 0.996)	0.957 (0.824, 0.985)	-2.204	0.100
MFI of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	2.65 (1.75, 3.38)	2.17 (1.70, 2.92)	-1.646	0.027
Percentage of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	0.986 (0.868, 0.996)	0.938 (0.823, 0.979)	-2.758	0.006
MFI of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	2.73 (1.73, 3.02)	2.10 (1.68, 2.54)	-2.668	0.008

All the data in Supplemental table 5 are represented as the median [IQR]. Abbreviations: IQR, interquartile Range; CD, cluster-of-differentiation; NK, natural killer; Treg, regulatory T; GR, Glucocorticoid receptor; MFI, mean fluorescence intensity.

Supplemental Table 6. The flow cytometry results of GR expression in the survivors and non-survivors.

	Survivors (n=20)	Non-survivors (n=65)	Z-value	P-value
Percentage of GR on B lymphocytes	0.904 (0.595, 0.976)	0.906 (0.657, 0.946)	-0.787	0.431
MFI of GR on B lymphocytes	1.92 (1.52, 2.54)	1.72 (1.51, 2.31)	-0.881	0.378
Percentage of GR on T lymphocytes	0.899 (0.778, 0.969)	0.913 (0.692, 0.951)	-1.057	0.291
MFI of GR on T lymphocytes	2.05 (1.67, 2.83)	1.91 (1.64, 2.46)	-1.031	0.303
Percentage of GR on NK cells	0.717 (0.292, 0.886)	0.556 (0.302, 0.823)	-0.756	0.449
MFI of GR on NK cells	1.54 (1.37, 2.09)	1.61 (1.34, 1.87)	-0.565	0.572
Percentage of GR on Tregs	0.780 (0.667, 0.849)	0.799 (0.576, 0.923)	-0.440	0.660
MFI of GR on Tregs	1.61 (1.48, 2.30)	1.77 (1.45, 2.27)	-0.005	0.996
Percentage of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	0.975 (0.876, 0.985)	0.957 (0.845, 0.987)	-0.617	0.538
MFI of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	2.08 (1.72, 3.35)	2.22 (1.71, 2.69)	-0.865	0.387
Percentage of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	0.963 (0.816, 0.977)	0.938 (0.834, 0.980)	-0.254	0.800
MFI of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	2.08 (1.68, 3.10)	2.11(1.71, 2.46)	-0.653	0.514

All the data in Supplemental table 6 are represented as the median [IQR]. Abbreviations: IQR, Interquartile Range; CD, Cluster-of-differentiation; NK, natural killer; Treg, regulatory T; GR, glucocorticoid receptor; MFI, mean fluorescence intensity.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	Supplemental Figure 1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-9, Supplemental Figure 1
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5,6,8,9
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5, 6, 8, Supplemental Figure 1
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	6-8
Study size	10	Explain how the study size was arrived at	Supplemental Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8, 11

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(b) Describe any methods used to examine subgroups and interactions	N/A
(c) Explain how missing data were addressed	8, 11
(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	11
(e) Describe any sensitivity analyses	

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60**Results**

Participants	13 *	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Supplemental Figure 1
		(b) Give reasons for non-participation at each stage	11, Supplemental Figure 1
		(c) Consider use of a flow diagram	Supplemental Figure 1
Descriptive data	14 *	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	9-11
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15 *	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	9-11
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-11, Electronic supplemental material
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A

Discussion

Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

1
2 available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at
3 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is
4 available at www.strobe-statement.org.
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Glucocorticoid receptor expression in patients with cardiac arrest in the early period after the return of spontaneous circulation: A prospective observational study

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1 **Glucocorticoid receptor expression in patients with cardiac arrest in the early**
2 **period after the return of spontaneous circulation: A prospective observational**
3 **study**

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17 **Keywords:** Cardiac arrest, glucocorticoid receptor, immunosuppression, cortisol

18 **Word count of the main text:** 3,335 words

23 Abstract

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

29 **Design:** Prospective observational study.

30 **Setting:** Emergency department.

31 **Participants:** Patients (85) in the early period of return of spontaneous circulation
32 (ROSC) after CA were admitted between October 2018 and October 2019. After a
33 physical examination, age- and sex-matched healthy individuals (40) were recruited for
34 the control group.

35 **Primary and secondary outcome measures:** GR expression and cell counts of
36 circulatory T and B lymphocytes, natural killer cells, and regulatory T (Treg) cells were
37 assessed. Plasma total cortisol and adrenocorticotrophic hormone (ACTH) levels were
38 also tested.

39 **Results:** All cell counts were lower, and plasma total cortisol levels were higher
40 ($P<0.001$) in patients who experienced CA than in the healthy control group. GR
41 expression in Treg cells and $CD3^+CD4^+$ T lymphocytes were not significantly different,
42 but the mean fluorescence intensity and GR expression in other cells were lower in
43 patients who experienced CA ($P<0.05$) than in the healthy control group. ACTH levels
44 were not different. There were no significant differences between survivors and non-

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4 45 survivors.

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6 46 **Conclusions:** This study revealed that GR expression and cell counts rapidly decreased,
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9 47 whereas plasma total cortisol levels increased in the early period after ROSC among
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12 48 patients who experienced CA. Our findings provide important information about GR
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15 49 level and function, and immunosuppressive status in these patients. Assessing GR
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17 50 expression in CA patients may help screening for those who are more sensitive to
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19 51 glucocorticoid therapy.
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23 24 25 53 **Strengths and limitations of this study**

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27 54 1. The study design will be single-center, prospective.
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30 55 2. This is the first study to evaluate the GR expression in the early period following
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32 56 ROSC among CA patients.
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35 57 3. Only CA patients in the early period following ROSC will be included, limiting the
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37 58 generalisability of the results.
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40 59 4. Decreased GR expression may affect the sensitivity of CA patients to GCs.
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43 60 5. Decreased GR expression may affect potential immune consequences of CA
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45 61 patients.
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49 50 51 63 **Introduction**

52
53 64 Cardiac arrest (CA) is a significant health problem globally; about 356,500 people
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55 65 experience medical emergencies due to CA in the United States, and over 544,000
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57 66 people die from sudden CA in China annually. [1, 2] The systemic ischemia-reperfusion
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4 67 response in patients who have experienced CA can present as post-cardiac arrest
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6 68 syndrome (PCAS) or systematic inflammatory response syndrome (SIRS), which
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9 69 increases the risk of multiple organ failure and infection and affects the inflammatory
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12 70 response and prognosis of patients after the return of spontaneous circulation (ROSC).
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14 71 [3-6]

17 72 CA is the most intense among acute stress events, which seriously affect the pituitary
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19 73 and adrenal axis function. [7] Studies have shown that abnormal cortisol levels and
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22 74 relative adrenocortical insufficiency after ROSC in patients who experienced CA are
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25 75 related to their prognosis. [8-11] However, the clinical application of glucocorticoids
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27 76 (GCs) is controversial. In the 2015 International Cardiopulmonary Resuscitation
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30 77 Guidelines, the routine use of GCs is not recommended for the resuscitation of patients
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33 78 with in-hospital or out-of-hospital CA. [12] Recent clinical studies have shown that
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36 79 early administration of corticosteroids after CA can improve the success rate of ROSC,
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38 80 nervous system functional outcome, and prognosis, which is speculated to be related to
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41 81 its influence on hemodynamics, and SIRS response, and other mechanisms. [12-17]
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43 82 Therefore, the role of GCs in the occurrence and development of PCAS needs to be
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46 83 studied further.

48 84 GCs combine with intracellular GC receptors (GRs) to exert anti-inflammatory and
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51 85 immunosuppressive effects and reduce the production and the release of inflammatory
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54 86 cytokines. [18, 19] The affinity of GRs to GCs in circulating monocytes is decreased in
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57 87 patients with acquired immunodeficiency syndrome. [20] The expression of GR alpha
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59 88 and beta in peripheral polymorphonuclear cells is decreased in patients with critical
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4 89 illness, [21] pediatric septic shock, and high serum cortisol levels. [22] However, no
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6 90 study has reported the GR expression after ROSC in patients who experienced CA.
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9 91 Previous studies have found that the counts of circulating B and T lymphocytes,
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11 92 regulatory T (Treg) cells, and monocytes and expression of human leukocyte antigen
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13 93 DR (HLA-DR) on circulatory monocytes and B and T lymphocytes are reduced. [23,
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15 94 24] Hence, this study aimed to investigate the relationship between GR expression and
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17 95 immune alteration in the early period after ROSC in patients who experienced CA by
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19 96 observing GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells,
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21 97 their cell counts, and total plasma cortisol and adrenocorticotrophic hormone (ACTH)
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23 98 levels.
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32 100 **Materials and methods**

33 101 **Study participants**

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37 102 This was an observational study conducted in the Emergency Department (ED).
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39 103 According to the 2015 International Cardiopulmonary Resuscitation Guidelines, [25]
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41 104 we enrolled patients in the early ROSC period after CA (both in-hospital and out-of-
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43 105 hospital CA) and were admitted to the ED between October 2018 and October 2019.
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46 106 The inclusion criteria were patients with CA > 6 and < 24 hours after ROSC, with a
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48 107 Glasgow coma score < 8. The exclusion criteria were (a) <18 years of age, (b) terminal
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50 108 stage of disease (such as cancer of any type, acquired immunodeficiency syndrome),
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52 109 (c) corticosteroid treatment within the past three months, (d) administration of
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54 110 corticosteroids, and (e) adrenal insufficiency. All patients were treated according to the
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111 2015 International Cardiopulmonary Resuscitation Consensus. [13] After a physical
112 examination, age- and sex-matched healthy individuals were recruited for the control
113 group.

114 **Data collection**

115 Data collection was performed according to the 2004 guidelines of the Utstein Style
116 template. [26] We collected data on demographics, resuscitation (initial heart rhythm,
117 ROSC time, and cumulative adrenaline [epinephrine] dose, and laboratory findings
118 routine blood cell counts, blood gas analysis, and blood biochemical tests performed >
119 6 h and < 24 h after ROSC). Acute Physiology and Chronic Health Evaluation
120 (APACHE) II and the Sequential Organ Failure Assessment (SOFA) were used to
121 determine disease severity. Residual blood samples from routine clinical tests or
122 physical health examinations in the morning were collected, maintained at 4 °C during
123 transport and storage, and used to determine GR expression in circulatory T and B
124 lymphocytes, NK cells, and Treg cells and their cell counts. The plasma was maintained
125 at -80 °C during storage and used to determine total cortisol and ACTH levels. During
126 follow-up, 28-day survival data were also collected. Supplemental Figure 1 shows the
127 workflow of this study.

128 **Outcome measures**

129 The primary outcomes of this study were GR expression and cell counts of T and B
130 cells, NK cells, and Treg cells, measured by flow cytometry. Venous blood samples
131 collected in ethylenediaminetetraacetic acid tubes, then used to measure GR expression
132 in T and B lymphocytes, NK cells, and Treg cells. Briefly, a 100- μ L peripheral blood

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4 133 sample was stained for 20 min with surface antibodies (CD3, CD4, CD8, CD19, CD16,
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7 134 CD56, CD25, and CD127) in a dark place. Erythrocytes were lysed for 15 min, and the
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10 135 debris was washed away. Before intracellular GR staining, surface-stained cells were
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12 136 fixed and permeabilized using the BD Transcription Factor Buffer Set (BD
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14 137 Pharmingen, San Diego, USA, Catalogue No. 562574). Monoclonal antibodies and
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17 138 their isotype controls were all purchased from BD Biosciences (San Jose, CA, USA).
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20 139 Details of all antibodies are shown in Supplemental Table 1. According to the
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22 140 manufacturer's recommendations, all antibodies and their isotype controls were used at
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25 141 a concentration of 1 μ L per 100 μ L of whole blood. Samples were measured using the
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27 142 Gallios flow cytometer (Beckman Coulter, Brea, CA, USA) and analyzed using Gallios
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30 143 Software version 1.0 (Beckman Coulter). The flow cytometer was periodically
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33 144 calibrated by an engineer. Cells were stained for 20 min; thresholds were defined using
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35 145 the manufacturer's recommended isotype controls. Representative plots and gating
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38 146 strategy from a single sample are shown in Supplemental Figure 2. T cells were gated
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40 147 by CD3⁺CD4⁺ or CD3⁺CD8⁺, B cells were gated by CD3⁻CD19⁺, NK cells were gated
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43 148 by CD16⁺CD56⁺, and Tregs were gated by CD4⁺CD25^{high}CD127^{low}. At least 10,000
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46 149 events were collected in the lymphocyte cell gate for each sample. Results are expressed
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49 150 as percentages and mean fluorescence intensity (MFI) values.

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51 151 Absolute CD3⁺ and CD4⁺ lymphocyte, NK cell, and Treg cell counts were obtained
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53 152 using Flow-Count fluorospheres (Beckman Coulter, Catalogue No. 7547053),
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56 153 according to the manufacturer's instructions. B, CD3⁺CD4⁺T, CD3⁺CD8⁺T, and Treg
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59 154 cell counts were calculated by their percentages in CD3⁺ or CD4⁺ lymphocytes
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4 155 multiplied by CD3⁺ or CD4⁺ lymphocyte counts.
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6 156 The secondary outcomes of this study were plasma total cortisol and ACTH levels
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9 157 after ROSC. Venous blood samples were collected in heparin anticoagulant tubes,
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12 158 centrifuged 10 min at 3000 rpm, and then stored at -80 °C. Plasma total cortisol
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14 159 (IMMULITE 2000 Cortisol, L2KCO2, UK) and ACTH (IMMULITE 2000 ACTH,
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16 160 L2KAC2, UK) levels were assayed using a chemiluminescent immunoassay on a
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19 161 Siemens automated analyzer (IMMULITE 2000 XPi; Siemens Healthcare Diagnostics,
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22 162 Erlangen, Germany). The equipment and reagents were calibrated by engineers before
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25 163 use. The lower detection limit of total cortisol was 2.00 ng/mL, and that of ACTH was
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27 164 5.00 pg/mL.
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30 165 **Statistical analyses**

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32 166 Data analysis was used in SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and
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35 167 sample size calculation in PASS15.0 software (NCSS, LLC, Kaysville, UT, USA). For
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38 168 normally distributed data, continuous variables are expressed as means with standard
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41 169 deviations. Since the data for total cortisol and ACTH levels had a skewed distribution,
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44 170 we compared our results with the natural logarithmic conversion values after adding 1
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46 171 (\ln [total cortisol+ 1], \ln [ACTH+ 1]). Measurement data with a skewed distribution are
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49 172 expressed as medians (25th and 75th percentiles). The Mann–Whitney U test was used
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52 173 to compare variables between groups. The qualitative parameters in the 2×2
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54 174 contingency table were used for analysis. All statistical tests were two-tailed, and a P-
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56 175 value of <0.05 was considered statistically significant.
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58 176 **Follow-up**

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177 Patients were classified into survivor and non-survivor groups according to the 28-
178 day survival endpoint. Those with all-cause mortality within the follow-up period were
179 considered non-survivors. If data were lost, the corresponding candidate was excluded.

180 **Patient and public involvement**

181 Patients and/or the public were not involved in the design, or conduct, or reporting,
182 or dissemination plans of this research.

184 **Results**

185 **Patient characteristics**

186 40 healthy individuals and 85 patients who experienced CA were analyzed. The
187 demographics and clinical characteristics of both groups are shown in Table 1. In this
188 study, acute cardiac and brain events were the main causes of CA, with those in the
189 latter category emanating from strokes. Other causes of CA included poisoning
190 (including carbon monoxide poisoning) and hypokalemia. Sex and age were not
191 significantly different between the CA and healthy control groups. The comparisons of
192 clinical characteristics of the survivor and non-survivor groups based on 28-day
193 survival are shown in Supplemental Table 2. The APACHE II and SOFA scores were
194 significantly different between the CA and healthy control groups ($P < 0.001$ for all) and
195 survivor and non-survivor groups ($P < 0.001$ and $P = 0.011$, respectively).

197 **Table 1. Patient Characteristics at Admission**

	Healthy Control	Successful Resuscitation
Characteristics	Group (n=40)	Group (n=85)
Age (years), median [IQR]	64.0 (54.3, 69.8)	65.0 (55.0, 74.0)
Male/Female (n)	23/17	58/27
Previous medical history, n (%)		
Hypertension	5 (12.5%)	38 (44.7%)
Diabetes	3 (7.5%)	27 (31.8%)
Coronary heart disease	2 (5.0%)	29 (34.1%)
Chronic lung disease	1 (2.5%)	9 (10.6%)
Chronic kidney disease	0	9 (10.6%)
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)
White cell count ($\times 10^9/L$), median [IQR]	5.81 (4.85, 6.53)	13.56 (10.84, 18.29)
APACHE II score, mean \pm SD	0	32.9 \pm 6.5
SOFA score, median [IQR]	0	11.5 (8.5, 14.0)
28-day mortality, n (%)		65 (76.5%)
28-day CPC 1–2, n (%)		14 (16.5%)

198 Abbreviations: IQR: interquartile range; ROSC: return of spontaneous circulation;

199 VF: ventricular fibrillation; VT: ventricular tachycardia; MAP: mean arterial pressure;

200 APACHE II: acute physiology and chronic health evaluation; SOFA: sequential

201 organ failure assessment; SD: standard deviation; CPC: cerebral performance
202 category.

203 **Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts after** 204 **ROSC**

205 The T and B lymphocyte, NK cell, and Treg cell counts were significantly lower after
206 ROSC in patients who experienced CA than in healthy controls ($P < 0.001$ for all).
207 Additionally, the $CD3^+CD4^+/T$ lymphocyte, $CD3^+CD8^+/T$ lymphocyte, and Treg
208 cell/ $CD4^+$ T lymphocyte ratios were significantly lower after ROSC in patients who
209 experienced CA than in healthy controls ($P < 0.001$ for all) (Fig. 1; Supplemental Table
210 3). However, there were no significant differences in these cell counts and ratios
211 between survivors ($n=20$) and non-survivors ($n=65$) ($P > 0.05$ for all) (Supplemental
212 Table 4).

213 **GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells after** 214 **ROSC**

215 The MFI and percentages of GR expression in B and T lymphocytes, NK cells, and
216 $CD3^+CD8^+$ T lymphocytes were significantly lower after ROSC in patients who
217 experienced CA than in healthy individuals ($P < 0.01$ for all) (Fig. 2A–D, G, H, K, L).
218 There were also significant reductions in the MFI in Treg cells and $CD3^+CD4^+$ T
219 lymphocytes ($P < 0.05$ for all) (Fig. 2E, I) but not in the percentages of GR expression
220 ($P > 0.05$ for all) (Fig. 2F, J; Supplemental Table 5). However, there were no significant
221 differences in the MFI and percentages of GR expression in these cells between
222 survivors and non-survivors ($P > 0.05$ for all) (Supplemental Table 6).

223 **Changes in plasma total cortisol and ACTH levels after ROSC**

224 We measured the plasma total cortisol and ACTH levels of the 40 healthy individuals
225 and 85 patients who experienced CA (two samples were excluded because their total
226 cortisol levels were not measured). Plasma total cortisol levels were significantly higher
227 in patients who experienced CA than in healthy controls ($P < 0.001$), but ACTH levels
228 were not (Fig. 3A, C). No significant differences in $\ln(\text{total cortisol}+1)$ and \ln
229 $(\text{ACTH}+1)$ values were observed between survivors and non-survivors ($P > 0.05$ for all)
230 (Fig. 3B, D).

232 **Discussion**

233 In this study, we examined the levels of GR expression and plasma corticosteroids
234 in patients with CA in the early period after ROSC. We found that GR expression in
235 circulatory T and B lymphocytes, NK cells, and Treg cells, cell counts and ratios in
236 patients with CA was significantly lower compared to that in controls. Furthermore,
237 plasma total cortisol levels in patients with CA were significantly higher compared to
238 the controls.

239 The ischemia-reperfusion response initiates an acute inflammatory response that
240 contributes to post-resuscitation shock after CA.[27] The immune response of patients
241 who experience CA is impaired, and the systemic inflammatory response increases. [6,
242 28] The T and B lymphocyte, NK cell, and Treg cell counts and $\text{CD3}^+\text{CD4}^+/\text{T}$,
243 $\text{CD3}^+\text{CD8}^+/\text{T}$, and Treg cell/ CD4^+ T lymphocyte ratios were significantly reduced after
244 ROSC. NK cells, which are special innate immune cells with cytotoxic functions

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4 245 similar to CD3⁺CD8⁺ T lymphocytes, mainly distinguish infected and stressed cells
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6 246 from healthy cells and eliminate intracellular infection and dysfunctional cells. [29, 30]
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9 247 T lymphocytes are also crucial because they function as adaptive immune cells to
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12 248 control and eliminate the infection. [29] Moreover, B and T lymphocytes mediate
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14 249 humoral and cellular immunity, respectively. This study was performed earlier and
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17 250 involved a more comprehensive assessment of the immune system of patients who
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20 251 experienced CA. Our findings more substantially supported the rapid emergence of
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22 252 immune dysfunction in these patients after ROSC than in previous reports.

23
24 253 The binding of GCs to GR inside different peripheral blood mononuclear cells
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27 254 (PBMC) leads to changes in the ability of cells to regulate apoptosis, proliferation, and
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30 255 activity, and GC-GR complexes limit the transcription (trans-repression) of
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33 256 inflammatory genes, including those encoding for proinflammatory cytokines.[31, 32]
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35
36 257 This study is the first to explore GR expression in circulating immune cells in patients
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38 258 who experienced CA after ROSC. We observed that GR expression in B and T
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41 259 lymphocytes, NK cells, and CD3⁺CD8⁺ T lymphocytes decreased significantly in
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44 260 patients who experienced CA, whereas the percentage of GR⁺ Treg cells and
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47 261 CD3⁺CD4⁺ T lymphocytes decreased slightly. Moreover, we observed a more
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50 262 significant decrease in the MFI of GR expression in Treg cells and CD3⁺CD4⁺ T
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53 263 lymphocytes but not in the percentage of GR expression. Previous studies have found
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56 264 decreased expression of GRs in peripheral polymorphonuclear cells in critically ill
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59 265 patients, [21] and antagonism to GRs aggravates viral and bacterial infections. [33]
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266 GCs induced upon infections help to maintain homeostasis and mitigate the life-

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4 267 threatening impact of sepsis on the host.[31] Although studies have reported that the
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6 268 use of GCs during and after CPR seems to confer benefits concerning ROSC rates and
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9 269 long-term survival, the evidence is scant. [13,18,34,35] Since cortisol signaling is
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11
12 270 mediated by GRs, we hypothesized that the differential responses of CA patients to GC
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15 271 may be related to their levels of GR expression. This study suggests that the decrease
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17 272 in intracellular GR expression in patients who experienced CA is one of the causes of
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19 273 GC resistance due to insufficient binding of GRs and GCs, GC insensitivity, and the
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22 274 inability of GCs to exert anti-inflammatory and immunosuppressive effects effectively.
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24
25 275 These findings may also explain why different results regarding the clinical application
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27 276 of GCs have been reported previously. Furthermore, it is vital to measure GR levels as
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30 277 sufficient expression of GR is essential for mediating adequate GC effects during and
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33 278 after CPR.

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35 279 We also found that the total plasma cortisol levels were significantly higher in
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37 280 patients who experienced CA, but ACTH levels were not. High levels of inflammatory
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40 281 cytokines inhibit ACTH release. [18] During critical illness, the body does not
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43 282 sufficiently metabolize cortisol. [36] In addition, the continuous increase in plasma
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46 283 cortisol levels may trigger the negative feedback pathway of the hypothalamic-
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49 284 pituitary-adrenal axis, inhibiting the release of ACTH and cortisol and eventually
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52 285 leading to adrenal insufficiency [37]. These factors may explain the opposite trends of
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55 286 plasma ACTH and cortisol levels in the patients included in this study and who
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58 287 experienced CA. Notably, this result suggests that low GR expression levels are not
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60 288 matched by high plasma total cortisol levels in patients who experienced CA. The

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4 289 dissociation between low GR expression and high cortisol implies an abnormal stress
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6 290 response. [38] Although systemic cortisol levels may be high, its availability is low
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9 291 during cardiac arrest. Previous studies have found that GC use during resuscitation may
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11 292 benefit patients who experience CA. [13-16] Possible reasons for this response may be
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14 293 that large doses of GCs given to CA patients may stimulate the function of GRs, or that
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17 294 GR expression or GC sensitivity was better in some patients. The probability of
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19 295 systemic inflammatory response and immunosuppression may also have been reduced
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22 296 in some CA patients. This study did not provide data on plasma GC levels and GR
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25 297 expression in a group of patients who were administered GCs and successfully
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28 298 resuscitated; therefore, further studies are required.

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32 **Limitations**

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35 301 Our study has several limitations. First, to assess changes, we only enrolled patients
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37 302 who experienced CA and had signs of systemic ischemic hypoxia, such as GCS <8 after
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40 303 ROSC. The patients were not stratified by age, sex, the occurrence of comorbidities, or
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42
43 304 mild systemic ischemic hypoxia. Second, since this was a preliminary observational
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46 305 study, we observed only early changes. A more relevant control group and dynamic
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49 306 observations obtained over a longer duration would be helpful to understand the
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51 307 significance of GR expression in evolving immunity during the clinical course of CA
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54 308 after ROSC. Third, the samples used in this study were from clinical laboratories; thus,
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57 309 plasma total cortisol and ACTH in the samples were at risk of degradation before we
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59 310 collected the samples. Finally, we did not discuss the changes in and roles of GR

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4 311 isoforms, free cortisol, and corticosteroid-binding globulin. Therefore, future studies
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6 312 on these aspects are warranted to better understand the immunosuppressive effects of
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9 313 ROSC among patients who experienced CA.

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11 314 In conclusion, this study revealed that GR expression, cell counts and ratios rapidly
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14 315 decreased, whereas plasma total cortisol levels increased, in the early period after
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17 316 ROSC among CA patients. These findings may provide important information about
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20 317 GR expression levels and function, and immunosuppressive status in these patients. The
21
22 318 assessment of GR expression in CA patients may help screening for those who are more
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24
25 319 sensitive to glucocorticoid therapy.

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29
30 321 **Acknowledgments:** We thank all the patients and their families who were enrolled in
31
32 322 this study and colleagues from the emergency department who provided support. And
33
34
35 323 we are grateful for the efforts of the staff for ongoing resuscitation in hospitals.

36
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38
39
40 325 YNY searched the literature and contributed to the experimental studies, data analysis,
41
42
43 326 and manuscript writing. ZRT, CCH, and LA collected and analyzed data. JBL and MRX
44
45
46 327 helped with the statistical analyses. All authors have read and approved the final
47
48 328 manuscript.

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50 329 **Competing interests:** All authors declare no competing interest associated with this
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53 330 project.

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55
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57
58 332 commercial or not-for-profit sectors.

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4 333 **Provenance and peer review:** Not commissioned; externally peer-reviewed.
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6 334 **Data sharing statement:** All data relevant to the study are included in the article or
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8
9 335 uploaded as supplementary information. Due to privacy and ethical concerns, data can
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11
12 336 not be shared.
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17 338 **Ethics statements**

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19 339 **Patient consent for publication:** Not applicable.
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22 340 **Ethics approval:** This study was approved by the Medical Ethics Committee of Beijing
23
24 341 Chaoyang Hospital (2013-KE-1). After successful resuscitation, informed consent was
25
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27 342 obtained from the families of the patients to enroll them in the study.
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344 **References**

- 345 [1] Myat A, Song KJ, Rea T. Out-of-hospital cardiac arrest: current concepts. *Lancet*,
346 Mar 10, 2018. DOI: 10.1016/S0140-6736(18)30472-0.
- 347 [2] Zhang S. Sudden cardiac death in China: current status and future perspectives.
348 *Europace*, Oct, 2015. DOI: 10.1093/europace/euv143.
- 349 [3] Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology,
350 pathophysiology, treatment, and prognostication. A Scientific Statement from the
351 international Liaison Committee on Resuscitation; the American Heart Association
352 Emergency cardiovascular Care Committee; the Council on Cardiovascular Surgery
353 and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the
354 Council on Clinical Cardiology; the Council on Stroke. *Resuscitation*, Dec, 2008. DOI:
355 10.1016/j.resuscitation.2008.09.017.
- 356 [4] Su CP, Wu JH, Yang MC, et al. Demographics and clinical features of
357 postresuscitation comorbidities in long-term survivors of out-of-hospital cardiac arrest:
358 A national follow-up study. *Biomed Res Int*, 2017. DOI: 10.1155/2017/9259182.
- 359 [5] Tsai MS, Chiang WC, Lee CC, et al. Infections in the survivors of out-of-hospital
360 cardiac arrest in the first 7 days. *Intensive Care Med*, May 31, 2005. DOI:
361 10.1007/s00134-005-2612-6.
- 362 [6] Adrie C, Adib-Conquy M, Laurent I, et al. Successful cardiopulmonary
363 resuscitation after cardiac arrest as a "sepsis-like" syndrome. *Circulation*, Jul 30, 2002.
364 DOI: 10.1161/01.cir.0000023891.80661.ad.
- 365 [7] Hall ED. Neuroprotective actions of glucocorticoid and nonglucocorticoid steroids

-
- 1
2
3
4 366 in acute neuronal injury. *Cell Mol Neurobiol*, Aug 13, 1993. DOI:
5
6 367 10.1007/BF00711581.
7
8
9 368 [8] de Jong MF, Beishuizen A, de Jong MJ et al. The pituitary-adrenal axis is activated
10
11 369 more in non-survivors than in survivors of cardiac arrest, irrespective of therapeutic
12
13 370 hypothermia. *Resuscitation*, Sep, 2008. DOI: 10.1016/j.resuscitation.2008.03.227.
14
15
16 371 [9] Mosaddegh R, Kianmehr N, Mahshidfar B et al. Serum cortisol level and adrenal
17
18 372 reserve as a predictor of patients' outcome after successful cardiopulmonary
19
20 373 resuscitation. *J Cardiovasc Thorac Res*, 2016. DOI: 10.15171/jcvtr.2016.12.
21
22
23 374 [10] Hékimian G, Baugnon T, Thuong M, et al. Cortisol levels and adrenal reserve after
24
25 375 successful cardiac arrest resuscitation. *Shock*, Aug, 2004. DOI:
26
27 376 10.1097/01.shk.0000132489.79498.c7.
28
29
30 377 [11] Tavakoli N, Bidari A, Shams Vahdati S. Serum Cortisol levels as a predictor of
31
32 378 neurologic survival in successfully resuscitated victims of cardiopulmonary arrest. *J*
33
34 379 *Cardiovasc Thorac Res*, 2012. DOI: 10.5681/jcvtr.2012.026.
35
36
37 380 [12] Soar J, Callaway CW, Aibiki M, et al. Resuscitation- Part 4: advanced life support:
38
39 381 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency
40
41 382 Cardiovascular Care Science with Treatment Recommendations. *Resuscitation*, Oct,
42
43 383 2015. DOI: 10.1016/j.resuscitation.2015.07.042.
44
45
46 384 [13] Mentzelopoulos SD, Malachias S, Chamos C, et al. Vasopressin, steroids, and
47
48 385 epinephrine and neurologically favorable survival after in-hospital cardiac arrest: a
49
50 386 randomized clinical trial. *JAMA*, Jul 17, 2013. DOI: 10.1001/jama.2013.7832.
51
52
53 387 [14] Tsai MS, Chuang PY, Yu PH, et al. Glucocorticoid use during cardiopulmonary
54
55
56
57
58
59
60

-
- 1
2
3
4 388 resuscitation may be beneficial for cardiac arrest. *Int J Cardiol*, Nov 1, 2016. DOI:
5
6 389 10.1016/j.ijcard.2016.08.017.
7
8
9 390 [15] Niimura T, Zamami Y, Koyama T, et al. Hydrocortisone administration was
10
11 391 associated with improved survival in Japanese patients with cardiac arrest. *Sci Rep*,
12
13 392 Dec 20, 2017. DOI: 10.1038/s41598-017-17686-3.
14
15
16 393 [16] Chalkias A, Xanthos T. Post-cardiac arrest syndrome: mechanisms and evaluation
17
18 394 of adrenal insufficiency. *World J Crit Care Med*, Feb 4, 2012. DOI:
19
20 395 10.5492/wjccm.v1.i1.4.
21
22
23 396 [17] Buddineni JP, Callaway C, Huang DT. Epinephrine, vasopressin and steroids for
24
25 397 in-hospital cardiac arrest: the right cocktail therapy? *Crit Care*, Jun 2, 2014. DOI:
26
27 398 10.1186/cc13903.
28
29
30 399 [18] Varvarousi G, Stefaniotou A, Varvaroussis D et al. Glucocorticoids as an emerging
31
32 400 pharmacologic agent for cardiopulmonary resuscitation. *Cardiovasc Drugs Ther*, Oct,
33
34 401 2014. DOI: 10.1007/s10557-014-6547-4.
35
36
37 402 [19] Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease.
38
39 403 *Trends Pharmacol Sci*, Sep, 2013. DOI: 10.1016/j.tips.2013.07.003.
40
41
42 404 [20] Norbiato G, Bevilacqua M, Vago T, et al. Cortisol resistance in acquired
43
44 405 immunodeficiency syndrome. *J Clin Endocrinol Metab*, Mar, 1992. DOI:
45
46 406 10.1210/jcem.74.3.1740494.
47
48
49 407 [21] Vassiliou AG, Floros G, Jahaj E, et al. Decreased glucocorticoid receptor
50
51 408 expression during critical illness. *Eur J Clin Invest*, Apr, 2019. DOI: 10.1111/eci.13073.
52
53
54 409 [22] Alder MN, Opoka AM, Wong HR. The glucocorticoid receptor and cortisol levels
55
56
57
58
59
60

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- 1
2
3
4 410 in pediatric septic shock. *Crit Care*, Sep 29, 2018. DOI: 10.1186/s13054-018-2177-8.
5
6 411 [23] Qi Z, Liu Q, Zhang Q et al. Overexpression of programmed cell death-1 and human
7
8 412 leucocyte antigen-DR on circulatory regulatory T cells in out-of-hospital cardiac arrest
9
10 413 patients in the early period after return of spontaneous circulation. *Resuscitation*, Sep,
11
12 414 2018. DOI: 10.1016/j.resuscitation.2018.06.023.
13
14 415 [24] Qi Z, An L, Liu B, et al. Patients with out-of-hospital cardiac arrest show decreased
15
16 416 human leucocyte antigen-DR expression on monocytes and B and T lymphocytes after
17
18 417 return of spontaneous circulation. *Scand J Immunol*, Oct, 2018. DOI:
19
20 418 10.1111/sji.12707.
21
22 419 [25] Perkins GD, Travers AH, Berg RA, et al. Resuscitation-Part 3: adult basic life
23
24 420 support and automated external defibrillation: 2015 International Consensus on
25
26 421 Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with
27
28 422 Treatment Recommendations. *Resuscitation*, Oct, 2015. DOI:
29
30 423 10.1016/j.resuscitation.2015.07.041.
31
32 424 [26] Jacobs I, Nadkarni V, Bahr J, et al. Cardiac arrest and cardiopulmonary
33
34 425 resuscitation outcome reports: update and simplification of the Utstein templates for
35
36 426 resuscitation registries. A statement for healthcare professionals from a task force of
37
38 427 the international liaison committee on resuscitation (American Heart Association,
39
40 428 European Resuscitation Council, Australian Resuscitation Council, New Zealand
41
42 429 Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart
43
44 430 Foundation, Resuscitation Council of Southern Africa). *Resuscitation*, Dec, 2004. DOI:
45
46 431 10.1016/j.resuscitation.2004.09.008.
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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- 1
2
3
4 432 [27] Lindner KH, Strohmenger HU, Ensinger H, Hetzel WD, Ahnefeld FW, Georgieff
5
6 433 M. Stress hormone response during and after cardiopulmonary resuscitation.
7
8
9 434 *Anesthesiology*, Oct, 1992. DOI: 10.1097/00000542-199210000-00008.
10
11
12 435 [28] Beurskens CJ, Horn J, de Boer AM, et al. Cardiac arrest patients have an impaired
13
14 436 immune response, which is not influenced by induced hypothermia. *Crit Care*, Jul 30,
15
16 437 2014. DOI: 10.1186/cc14002.
17
18
19 438 [29] Lanier LL. NK cell recognition. *Annu Rev Immunol*, 2005. DOI:
20
21 439 10.1146/annurev.immunol.23.021704.115526.
22
23
24 440 [30] Vivier E, Tomasello E, Baratin M et al. Functions of natural killer cells. *Nat*
25
26 441 *Immunol*, May, 2008. DOI: 10.1038/ni1582.
27
28
29 442 [31] Zen M, Canova M, Campana C, et al. The kaleidoscope of glucocorticoid effects on
30
31 443 immune system. *Autoimmun Rev*, Apr, 2011. DOI: 10.1016/j.autrev.2010.11.009.
32
33
34 444 [32] Vandewalle J, Libert C. Glucocorticoids in Sepsis: To be or not to be. *Front*
35
36 445 *Immunol*, Jul 21, 2020. DOI: 10.3389/fimmu.2020.01318.
37
38
39 446 [33] Webster JI, Sternberg EM. Role of the hypothalamic-pituitary-adrenal axis,
40
41 447 glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial
42
43 448 and viral products. *J Endocrinol*, May, 2004. DOI: 10.1677/joe.0.1810207.
44
45
46 449 [34] Andersen LW, Isbye D, Kjærgaard J, et al. Effect of Vasopressin and
47
48 450 methylprednisolone vs placebo on return of spontaneous circulation in patients with In-
49
50 451 hospital cardiac arrest: A randomized clinical trial. *JAMA*, Oct 26, 2021. DOI:
51
52 452 10.1001/jama.2021.16628.
53
54
55 453 [35] Smithline H, Rivers E, Appleton T, Nowak R. Corticosteroid supplementation
56
57
58
59
60

-
- 1
2
3
4 454 during cardiac arrest in rats. Resuscitation, Jun, 1993. DOI: 10.1016/0300-
5
6 455 9572(93)90123-8.
7
8
9 456 [36] Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during
10
11 457 critical illness. N Engl J Med, Apr 18, 2013. DOI: 10.1056/NEJMoa1214969.
12
13
14 458 [37] Peeters B, Langouche L, Van den Berghe G. Adrenocortical stress response during
15
16 459 the course of critical illness. Compr Physiol, Dec 12, 2017. DOI:
17
18 460 10.1002/cphy.c170022.
19
20
21
22 461 [38] Vassiliou AG, Stamogiannos G, Jahaj E, et al. Longitudinal evaluation of
23
24 462 glucocorticoid receptor alpha/beta expression and signaling, adrenocortical function
25
26 463 and cytokines in critically ill steroid-free patients. Mol Cell Endocrinol, Feb 5, 2020.
27
28 464 DOI: 10.1016/j.mce.2019.110656.
29
30
31
32
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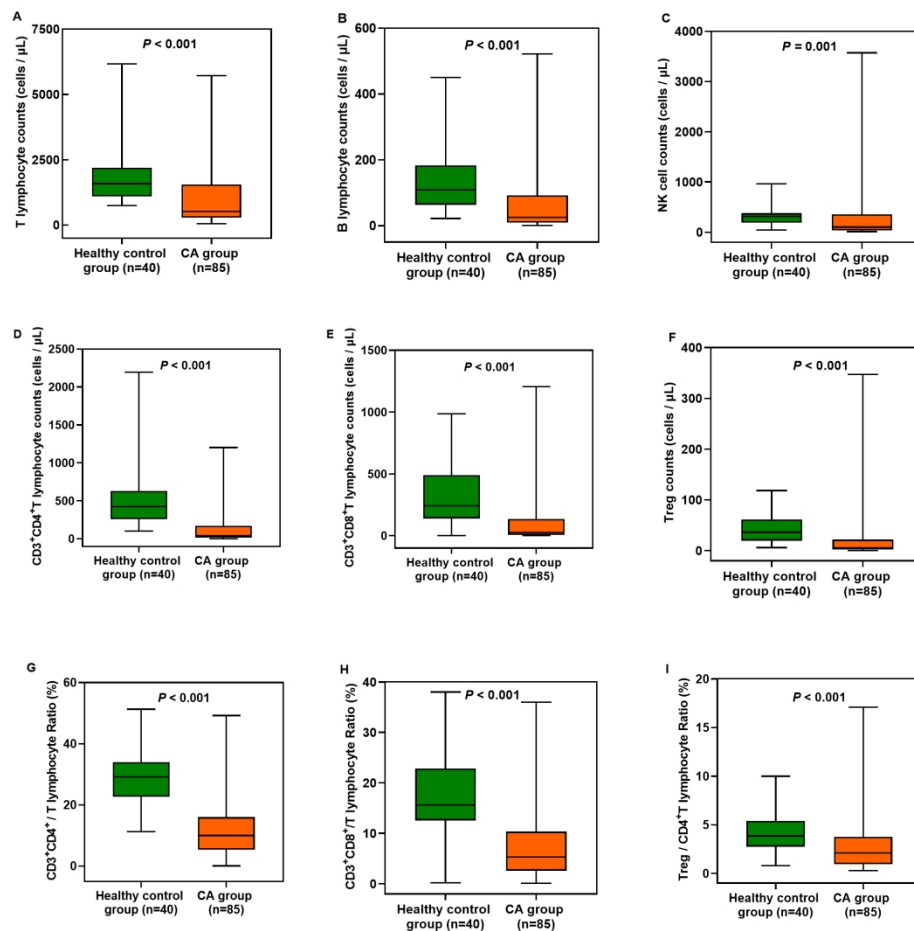
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Figure legends

Fig. 1. Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, 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; CD, cluster-of-differentiation; NK, natural killer; Treg, regulatory T.

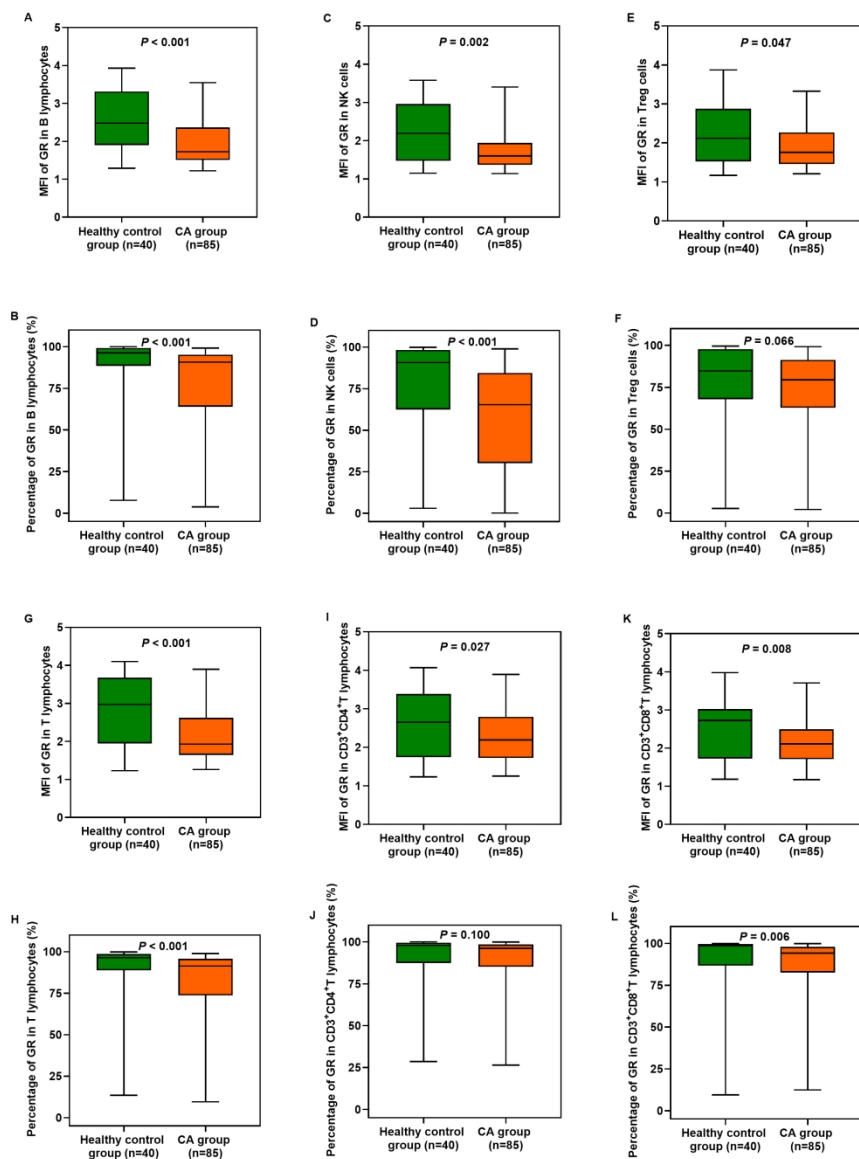
Fig. 2. Expression of GRs in circulatory T and B lymphocytes, NK cells, and Treg cells in the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group (P<0.05). CA, cardiac arrest; CD, cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC, return of spontaneous circulation; Treg, regulatory T.

Fig. 3. (A, B) Plasma total cortisol and ACTH levels (the natural logarithmic conversion values after adding 1) after ROSC in the healthy control group and CA group. (C, D) 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.



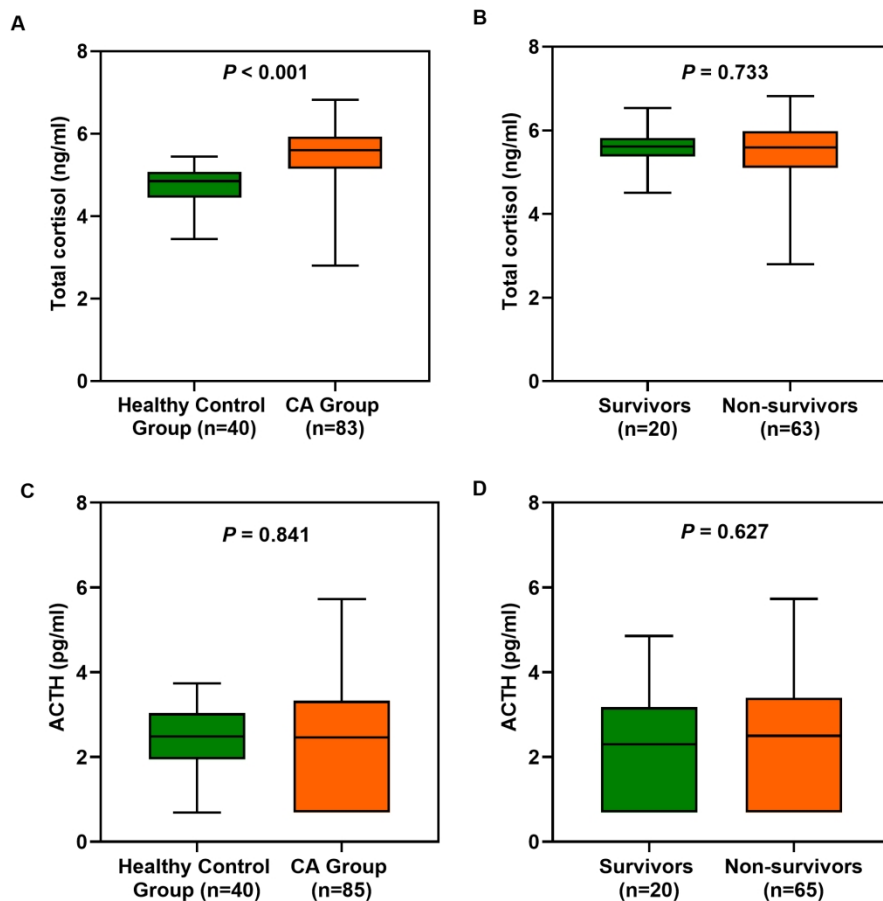
Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and CD3+CD4+/T, CD3+CD8+/T, and Treg/CD4+T lymphocyte ratios between the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group ($P < 0.001$). CA, cardiac arrest;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

187x183mm (300 x 300 DPI)



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; CD, cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC, return of spontaneous circulation; Treg, regulatory T.

199x256mm (300 x 300 DPI)



(A, B) Plasma total cortisol and ACTH levels (the natural logarithmic conversion values after adding 1) after ROSC in the healthy control group and CA group. (C, D) 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.

185x178mm (300 x 300 DPI)

Electronic supplemental material**Glucocorticoid receptor expression in patients with cardiac arrest in the early period after the return of spontaneous circulation: A prospective observational study**

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Contents

Supplemental Figure 1

Supplemental Figure 2

Supplemental Table 1

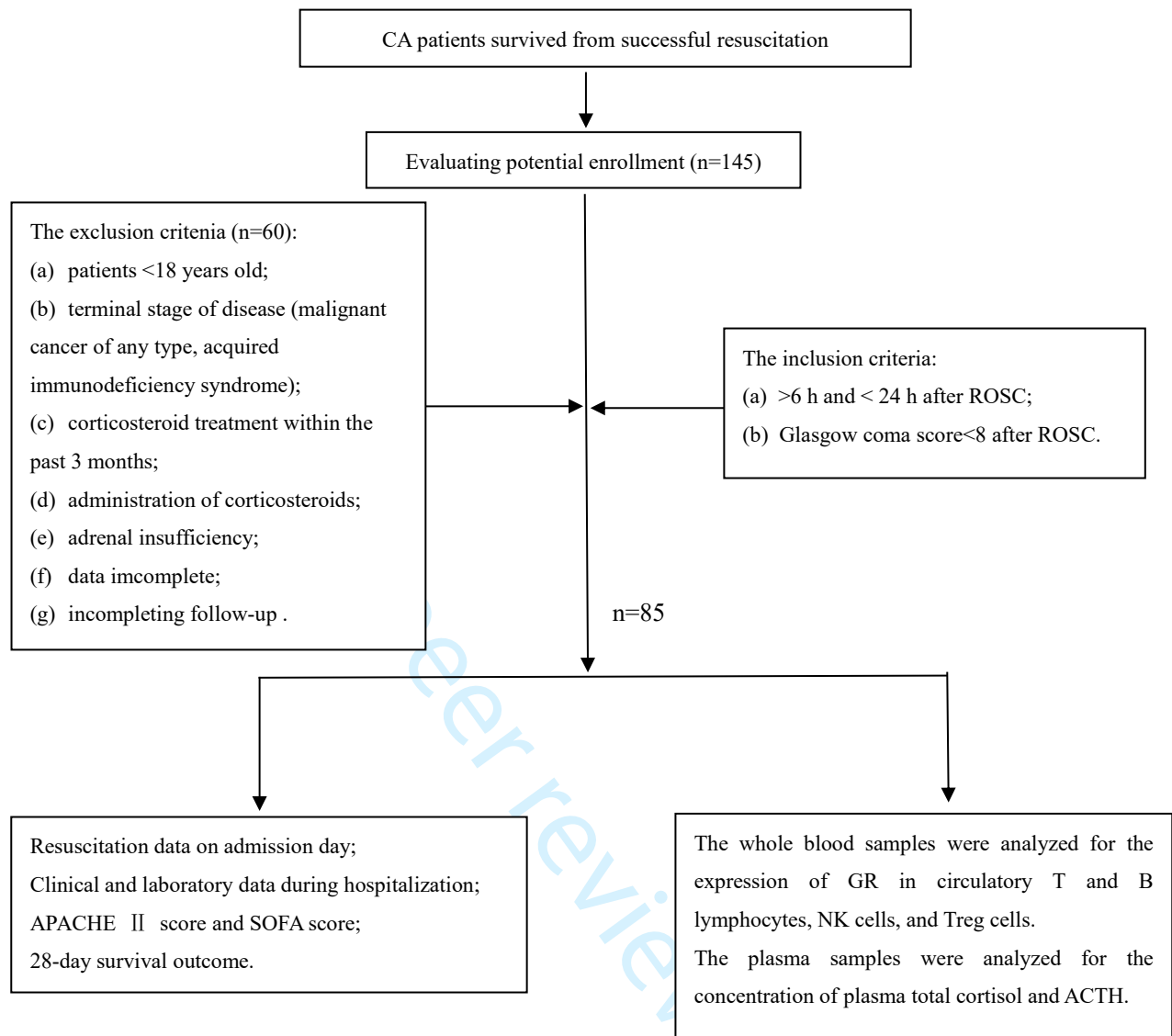
Supplemental Table 2

Supplemental Table 3

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Supplemental Table 6



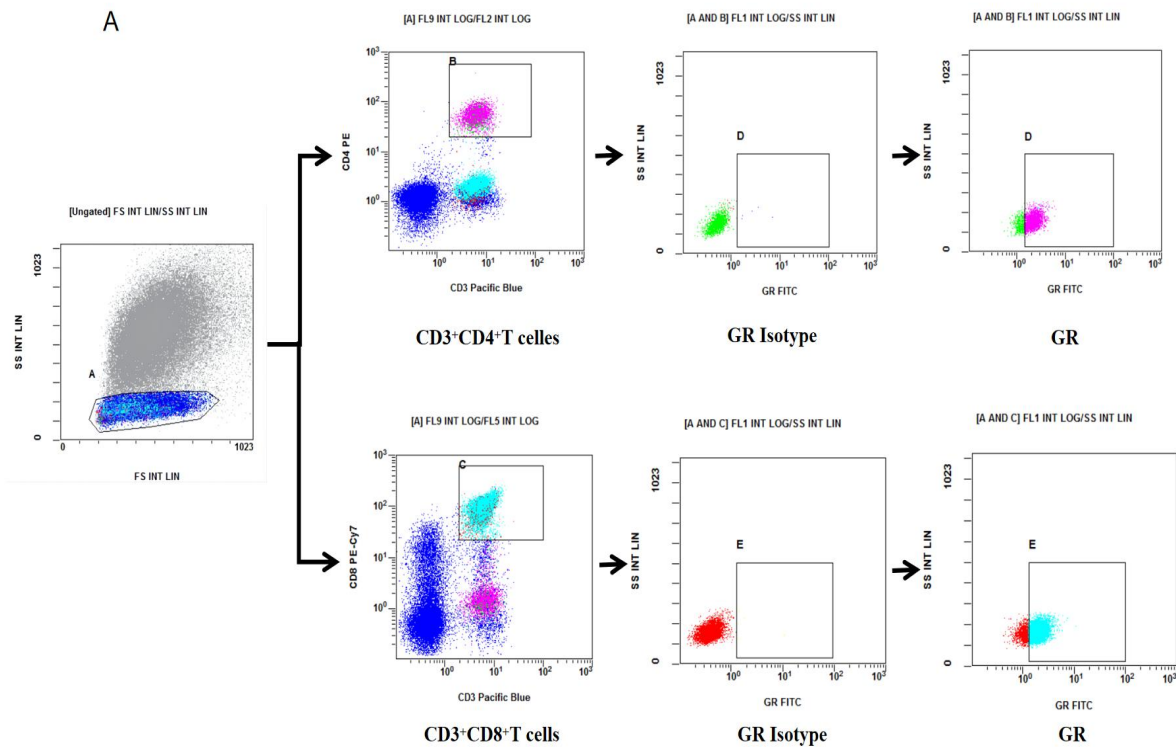
Supplemental Figure 1. The flow chart of the study.

Abbreviations: CA, cardiac arrest; ROSC, return of spontaneous circulation; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; GR, glucocorticoid receptor; Treg, regulatory T; ACTH, adrenocorticotrophic hormone.

Supplemental Figure 2. Representative plots and gating strategies for analyzing glucocorticoid receptor (GR) in the whole blood.

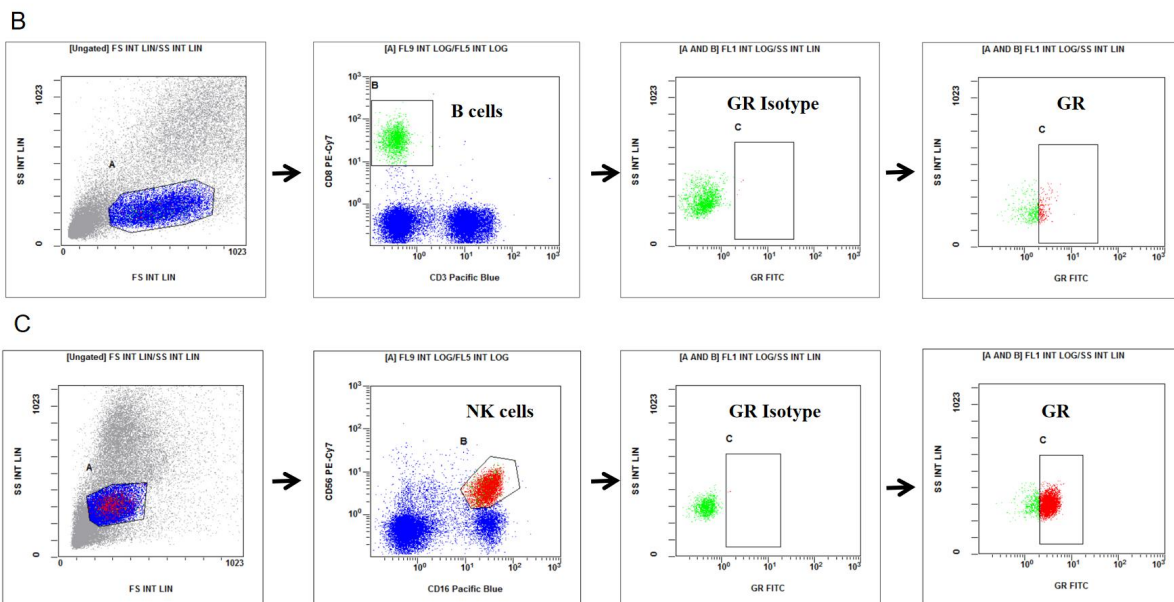
GR expression levels were determined on T cells, B cells, NK cells, and T regulatory (Treg) cells. Single cells were gated from all cellular events (FSC/SSC gate). B cells were identified as $CD3^+CD19^+$ cells. NK cells were identified as $CD16^+56^+$ cells. T cells were identified as $CD3^+CD4^+$ T cells and $CD3^+CD8^+$ T cells. Treg cells were identified as $CD4^+CD25^{\text{high}}CD127^{\text{low}}$.

A. Expression of GR on T cells

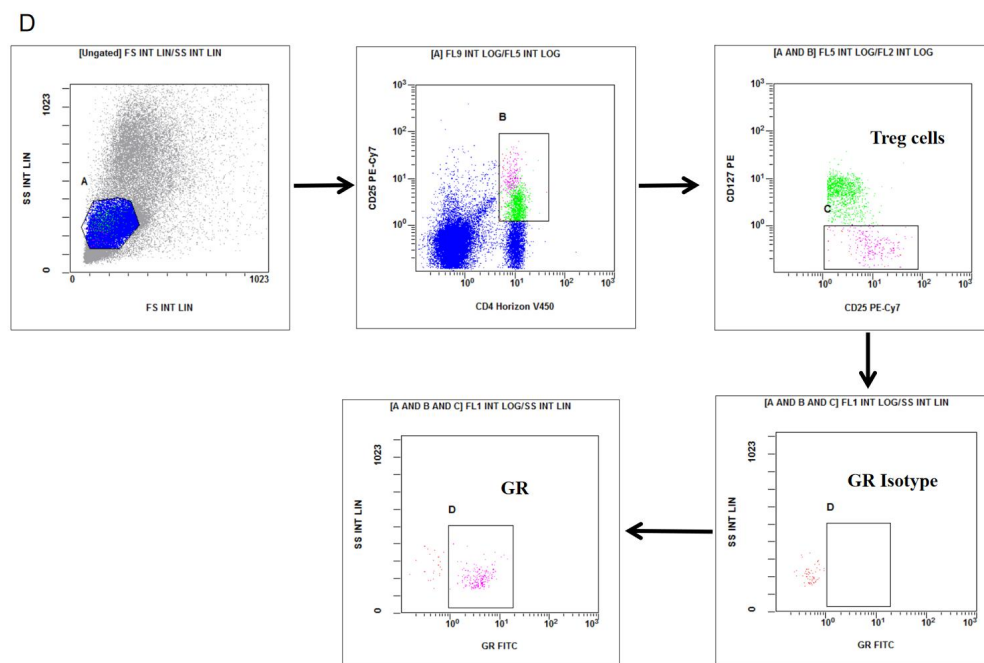


B. Expression of GR on B cells

C. Expression of GR on NK cells



D. Expression of GR on Treg cells



Supplemental Table 1. Details of antibodies for flow cytometry.

Antigen	Catalog Number	Fluorescein Conjugate	Source
CD3	558117	Pacific Blue	BD Pharmingen ^a
CD4	555347	PE	BD Pharmingen
CD4	560345	Horizon V450	BD Pharmingen
CD8	557746	PE-Cy7	BD Pharmingen
CD19	557835	PE-Cy7	BD Pharmingen
CD16	558122	Pacific Blue	BD Pharmingen
CD56	557747	PE-Cy7	BD Pharmingen
CD25	557741	PE-Cy7	BD Pharmingen
CD127	557938	PE	BD Pharmingen
GR	MCA2469F	FITC	Bio-Rad ^b
Mouse IgG1 Isotype	MCA928F	FITC	Bio-Rad
Mouse IgG1, κ Isotype	557872	PE-Cy7	BD Pharmingen
Mouse IgG1, κ Isotype	554680	PE	BD Pharmingen
Mouse IgG1, κ Isotype	558120	Pacific Blue	BD Pharmingen

^a BD Pharmingen, San Diego, USA; ^b Bio-Rad AbD Serotec, Oxford, UK.

Abbreviations: CD, cluster-of-differentiation; PE, phycoerythrin; FITC, fluorescein isothiocyanate; GR, glucocorticoid receptor; Ig: immunoglobulin.

Supplemental Table 2. Characteristics of CA survivors and non-survivors on admission.

	Survivors (n=20)	Non-survivors (n=65)
Age (years), median [IQR]	59.0 (53.3, 72.8)	66.0 (59.0, 75.5)
Male/Female (n)	12/8	46/19
Cardiac arrest cause (n, %)		
Cardiac	10 (50.0%)	24 (36.9%)
Non-Cardiac	10 (50.0%)	41 (63.1%)
Initial resuscitation		
Time to ROSC (min), median [IQR]	15.0 (7.3, 26.0)	20.0 (15.0, 30.0)
Adrenaline (mg), median [IQR]	1.0 (0.0, 3.0)	2.0 (0.0, 5.0)
Initial rhythm VF/VT, n (%)	11 (55.0%)	19 (29.2%)
MAP (mmHg), median [IQR]	89.9 (70.5, 104.9)	70.7 (50.0, 93.5)
White cell count ($\times 10^9/L$), median [IQR]	12.40 (6.98, 18.76)	13.80 (11.67, 18.20)
Lactate (mmol/L), median [IQR]	3.50 (1.33, 7.05)	7.50 (3.80, 11.20)
APACHE II score, mean \pm SD	27.8 \pm 6.6	34.4 \pm 5.6
SOFA score, median [IQR]	9.0 (7.3, 11.8)	12.0 (9.0, 15.0)

Data are presented as mean \pm SD or interquartile range (IQR) as appropriate. 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.

Supplemental Table 3. The flow cytometry results of cell counts and ratios of the healthy control group and successful resuscitation group

	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)	Z-value	P-value
T lymphocyte count (cells / μ L)	1586.0 (1101.5, 2192.5)	514.0 (287.5, 1555.0)	-4.515	<0.001
NK cell count (/ μ L)	311.5 (191.0, 378.8)	101.0 (36.0, 351.5)	-3.332	0.001
B lymphocyte count (/ μ L)	109.3 (63.7, 183.3)	25.7 (9.4, 92.3)	-5.076	<0.001
Treg count (/ μ L)	0.259 (0.095, 0.516)	0.233 (0.135, 0.488)	-5.518	<0.001
Treg / CD4 ⁺ T lymphocyte Ratio	0.039 (0.028, 0.054)	0.021 (0.010, 0.038)	-4.418	<0.001
CD3 ⁺ CD4 ⁺ T lymphocyte count (/ μ L)	421.7 (258.6, 627.4)	38.9 (17.6, 168.3)	-6.256	<0.001
CD3 ⁺ CD4 ⁺ / T lymphocyte Ratio	0.292 (0.227, 0.340)	0.100 (0.054, 0.160)	-7.066	<0.001
CD3 ⁺ CD8 ⁺ T lymphocyte count (/ μ L)	241.1 (139.5, 488.6)	26.3 (7.2, 135.9)	-5.287	<0.001
CD3 ⁺ CD8 ⁺ / T lymphocyte Ratio	0.157 (0.126, 0.229)	0.053 (0.026, 0.104)	-5.719	<0.001

All the data in Supplemental table 3 are represented as the median [IQR]; IQR: Interquartile Range; CD: cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; Treg, regulatory T.

Supplemental Table 4. The flow cytometry results of cell counts and ratios of the CA patients on admission based on 28-day survival

	Survivors (n=20)	Non-survivors (n=65)	Z-value	P-value
T lymphocyte count (/μL)	502.0 (353.8, 1199.8)	514.0 (282.5, 1891.0)	-0.186	0.852
NK cell count (/μL)	167.0 (29.8, 309.3)	100.0 (36.0, 404.0)	-0.218	0.828
B lymphocyte count (/μL)	38.6 (15.7, 103.5)	19.2 (7.1, 65.7)	-0.632	0.527
Tregs count (/μL)	0.318 (0.145, 0.552)	0.212 (0.128, 0.479)	-0.611	0.396
Treg / CD4 ⁺ T lymphocyte Ratio	0.025 (0.009, 0.043)	0.021 (0.010, 0.034)	-0.498	0.619
CD3 ⁺ CD4 ⁺ T lymphocyte count (/μL)	55.1 (32.4, 228.0)	38.0 (16.0, 168.1)	-0.850	0.396
CD3 ⁺ CD4 ⁺ / T lymphocyte Ratio	0.118 (0.070, 0.236)	0.097 (0.049, 0.142)	-1.565	0.118
CD3 ⁺ CD8 ⁺ T lymphocyte count (/μL)	25.4 (12.5, 96.2)	26.3 (6.3, 138.8)	-0.021	0.983
CD3 ⁺ CD8 ⁺ / T lymphocyte Ratio	0.054 (0.033, 0.104)	0.053 (0.025, 0.104)	-0.187	0.852

All the data in Supplemental table 4 are represented as the median [IQR]; IQR: Interquartile Range; CD: cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; Treg, regulatory T.

Supplemental Table 5. The flow cytometry results of GR expression in the CA group and successful resuscitation group.

	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)	Z-value	P-value
Percentage of GR on B lymphocytes	0.963 (0.885, 0.992)	0.896 (0.605, 0.949)	-3.742	<0.001
MFI of GR on B lymphocytes	2.48 (1.91, 3.31)	1.73 (1.50, 2.37)	-3.980	<0.001
Percentage of GR on T lymphocytes	0.964 (0.889, 0.986)	0.900 (0.703, 0.955)	-3.755	<0.001
MFI of GR on T lymphocytes	2.98(1.95, 3.68)	1.92 (1.36, 1.99)	-3.853	<0.001
Percentage of GR on NK cells	0.907 (0.624, 0.983)	0.611 (0.306, 0.840)	-3.792	<0.001
MFI of GR on NK cells	2.19 (1.48, 2.96)	1.60 (1.36, 1.99)	-3.171	0.002
Percentage of GR on Treg cells	0.848 (0.680, 0.978)	0.784 (0.589, 0.911)	-1.837	0.066
MFI of GR on Treg cells	2.12 (1.53, 2.88)	1.76 (1.44, 2.30)	-1.990	0.047
Percentage of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	0.980 (0.874, 0.996)	0.957 (0.824, 0.985)	-2.204	0.100
MFI of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	2.65 (1.75, 3.38)	2.17 (1.70, 2.92)	-1.646	0.027
Percentage of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	0.986 (0.868, 0.996)	0.938 (0.823, 0.979)	-2.758	0.006
MFI of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	2.73 (1.73, 3.02)	2.10 (1.68, 2.54)	-2.668	0.008

All the data in Supplemental table 5 are represented as the median [IQR]. Abbreviations: IQR, interquartile range; CD, cluster-of-differentiation; NK, natural killer; Treg, regulatory T; GR, Glucocorticoid receptor; MFI, mean fluorescence intensity.

Supplemental Table 6. The flow cytometry results of GR expression in the survivors and non-survivors.

	Survivors (n=20)	Non-survivors (n=65)	Z-value	P-value
Percentage of GR on B lymphocytes	0.904 (0.595, 0.976)	0.906 (0.657, 0.946)	-0.787	0.431
MFI of GR on B lymphocytes	1.92 (1.52, 2.54)	1.72 (1.51, 2.31)	-0.881	0.378
Percentage of GR on T lymphocytes	0.899 (0.778, 0.969)	0.913 (0.692, 0.951)	-1.057	0.291
MFI of GR on T lymphocytes	2.05 (1.67, 2.83)	1.91 (1.64, 2.46)	-1.031	0.303
Percentage of GR on NK cells	0.717 (0.292, 0.886)	0.556 (0.302, 0.823)	-0.756	0.449
MFI of GR on NK cells	1.54 (1.37, 2.09)	1.61 (1.34, 1.87)	-0.565	0.572
Percentage of GR on Tregs	0.780 (0.667, 0.849)	0.799 (0.576, 0.923)	-0.440	0.660
MFI of GR on Tregs	1.61 (1.48, 2.30)	1.77 (1.45, 2.27)	-0.005	0.996
Percentage of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	0.975 (0.876, 0.985)	0.957 (0.845, 0.987)	-0.617	0.538
MFI of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	2.08 (1.72, 3.35)	2.22 (1.71, 2.69)	-0.865	0.387
Percentage of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	0.963 (0.816, 0.977)	0.938 (0.834, 0.980)	-0.254	0.800
MFI of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	2.08 (1.68, 3.10)	2.11(1.71, 2.46)	-0.653	0.514

All the data in Supplemental table 6 are represented as the median [IQR]. Abbreviations: IQR, Interquartile Range; CD, Cluster-of-differentiation; NK, natural killer; Treg, regulatory T; GR, glucocorticoid receptor; MFI, mean fluorescence intensity.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	Supplemental Figure 1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-9, Supplemental Figure 1
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5,6,8,9
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5, 6, 8, Supplemental Figure 1
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 8
Bias	9	Describe any efforts to address potential sources of bias	6-8
Study size	10	Explain how the study size was arrived at	Supplemental Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8, 11

(b) Describe any methods used to examine subgroups and interactions	N/A
(c) Explain how missing data were addressed	8, 11
(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	11
(e) Describe any sensitivity analyses	

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Results			
Participants	13 *	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Supplementa l Figure 1
		(b) Give reasons for non-participation at each stage	9,12, Supplementa l Figure 1
		(c) Consider use of a flow diagram	Supplementa l Figure 1
Descriptive data	14 *	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	Supplementa l Figure 1
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15 *	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	9-12
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-12, Electronic supplemental material
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

1
2 available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at
3 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is
4 available at www.strobe-statement.org.
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Glucocorticoid receptor expression in patients with cardiac arrest in the early period after the return of spontaneous circulation: A prospective observational single-center study

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1 **Glucocorticoid receptor expression in patients with cardiac arrest in the early**
2 **period after the return of spontaneous circulation: A prospective observational**
3 **single-center study**

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17 **Keywords:** Cardiac arrest, glucocorticoid receptor, immunosuppression, cortisol

18 **Word count of the main text:** 3,437 words

23 Abstract

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

29 **Design:** Prospective observational study.

30 **Setting:** Emergency department.

31 **Participants:** Patients (85) in the early period of return of spontaneous circulation
32 (ROSC) after CA were admitted between October 2018 and October 2019. After a
33 physical examination, age- and sex-matched healthy individuals (40) were recruited for
34 the control group.

35 **Primary and secondary outcome measures:** GR expression and cell counts of
36 circulatory T and B lymphocytes, natural killer cells, and regulatory T (Treg) cells were
37 assessed. Plasma total cortisol and adrenocorticotrophic hormone (ACTH) levels were
38 also tested.

39 **Results:** All cell counts were lower, and plasma total cortisol levels were higher
40 ($P<0.001$) in patients who experienced CA than in the healthy control group. GR
41 expression in Treg cells and $CD3^+CD4^+$ T lymphocytes were not significantly different,
42 but the mean fluorescence intensity and GR expression in other cells were lower in
43 patients who experienced CA ($P<0.05$) than in the healthy control group. ACTH levels
44 were not different. There were no significant differences between survivors and non-

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4 45 survivors.

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6 46 **Conclusions:** This study revealed that GR expression and cell counts rapidly decreased,
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9 47 whereas plasma total cortisol levels increased in the early period after ROSC among
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12 48 patients who experienced CA. Our findings provide important information about GR
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15 49 level and function, and immunosuppressive status in these patients. Assessing GR
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17 50 expression in CA patients may help screening for those who are more sensitive to
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19 51 glucocorticoid therapy.
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23 24 25 53 **Strengths and limitations of this study**

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27 54 1. The study was designed as single-center, prospective study.
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30 55 2. This is the first study to evaluate the GR expression in the early period following
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33 56 ROSC among CA patients.
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35 57 3. We only studied the GR expression of CA patients in the early period following
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38 58 ROSC; therefore, our results cannot be extrapolated to time points beyond 24 hours.
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40 59 4. Decreased GR expression may affect the sensitivity of CA patients to GCs.
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43 60 5. Decreased GR expression may affect potential immune consequences of CA
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45 61 patients.
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49 50 51 63 **Introduction**

52
53 64 Cardiac arrest (CA) is a significant health problem globally; about 356,500 people
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56 65 experience medical emergencies due to CA in the United States, and over 544,000
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59 66 people die from sudden CA in China annually. [1, 2] The systemic ischemia-reperfusion
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4 67 response in patients who have experienced CA can present as post-cardiac arrest
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6 68 syndrome (PCAS) or systematic inflammatory response syndrome (SIRS), which
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9 69 increases the risk of multiple organ failure and infection and affects the inflammatory
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12 70 response and prognosis of patients after the return of spontaneous circulation (ROSC).
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14 71 [3-6]

17 72 CA is the most intense among acute stress events, which seriously affect the pituitary
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19 73 and adrenal axis function. [7] Studies have shown that abnormal cortisol levels and
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22 74 relative adrenocortical insufficiency after ROSC in patients who experienced CA are
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25 75 related to their prognosis. [8-11] However, the clinical application of glucocorticoids
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27 76 (GCs) is controversial. In the 2015 International Cardiopulmonary Resuscitation
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30 77 Guidelines, the routine use of GCs is not recommended for the resuscitation of patients
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33 78 with in-hospital or out-of-hospital CA. [12] Recent clinical studies have shown that
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36 79 early administration of corticosteroids after CA can improve the success rate of ROSC,
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38 80 nervous system functional outcome, and prognosis, which is speculated to be related to
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41 81 its influence on hemodynamics, and SIRS response, and other mechanisms. [12-17]
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43 82 Therefore, the role of GCs in the occurrence and development of PCAS needs to be
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46 83 studied further.

48 84 GCs combine with intracellular GC receptors (GRs) to exert anti-inflammatory and
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51 85 immunosuppressive effects and reduce the production and the release of inflammatory
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54 86 cytokines. [18, 19] The affinity of GRs to GCs in circulating monocytes is decreased in
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57 87 patients with acquired immunodeficiency syndrome. [20] The expression of GR alpha
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59 88 and beta in peripheral polymorphonuclear cells is decreased in patients with critical
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4 89 illness, [21] pediatric septic shock, and high serum cortisol levels. [22] However, no
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6 90 study has reported the GR expression after ROSC in patients who experienced CA.
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9 91 Previous studies have found that the counts of circulating B and T lymphocytes,
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11 92 regulatory T (Treg) cells, and monocytes and expression of human leukocyte antigen
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13 93 DR (HLA-DR) on circulatory monocytes and B and T lymphocytes are reduced. [23,
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15 94 24] Hence, this study aimed to investigate the relationship between GR expression and
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17 95 immune alteration in the early period after ROSC in patients who experienced CA by
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19 96 observing GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells,
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21 97 their cell counts, and total plasma cortisol and adrenocorticotrophic hormone (ACTH)
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23 98 levels.
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32 **Materials and methods**

33 **Study participants**

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37 102 This was an observational study conducted in the Emergency Department (ED).
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39 103 According to the 2015 International Cardiopulmonary Resuscitation Guidelines, [25]
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41 104 we enrolled patients in the early ROSC period after CA (both in-hospital and out-of-
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43 105 hospital CA) and were admitted to the ED between October 2018 and October 2019.
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47 106 The inclusion criteria were patients with CA > 6 and < 24 hours after ROSC, with a
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49 107 Glasgow coma score < 8. The exclusion criteria were (a) <18 years of age, (b) terminal
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51 108 stage of disease (such as cancer of any type, acquired immunodeficiency syndrome),
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53 109 (c) corticosteroid treatment within the past three months, (d) administration of
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55 110 corticosteroids, and (e) adrenal insufficiency. All patients were treated according to the
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111 2015 International Cardiopulmonary Resuscitation Consensus. [13] After a physical
112 examination, age- and sex-matched healthy individuals were recruited for the control
113 group.

114 **Data collection**

115 Data collection was performed according to the 2004 guidelines of the Utstein Style
116 template. [26] We collected data on demographics, resuscitation (initial heart rhythm,
117 ROSC time, and cumulative adrenaline [epinephrine] dose, and laboratory findings
118 routine blood cell counts, blood gas analysis, and blood biochemical tests performed >
119 6 h and < 24 h after ROSC). Acute Physiology and Chronic Health Evaluation
120 (APACHE) II and the Sequential Organ Failure Assessment (SOFA) were used to
121 determine disease severity. Residual blood samples from routine clinical tests or
122 physical health examinations in the morning were collected, maintained at 4 °C during
123 transport and storage, and used to determine GR expression in circulatory T and B
124 lymphocytes, NK cells, and Treg cells and their cell counts. The plasma was maintained
125 at -80 °C during storage and used to determine total cortisol and ACTH levels. During
126 follow-up, 28-day survival data were also collected. Supplemental Figure 1 shows the
127 workflow of this study.

128 **Outcome measures**

129 The primary outcomes of this study were GR expression and cell counts of T and B
130 cells, NK cells, and Treg cells, measured by flow cytometry. Venous blood samples
131 collected in ethylenediaminetetraacetic acid tubes, then used to measure GR expression
132 in T and B lymphocytes, NK cells, and Treg cells. Briefly, a 100- μ L peripheral blood

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4 133 sample was stained for 20 min with surface antibodies (CD3, CD4, CD8, CD19, CD16,
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6 134 CD56, CD25, and CD127) in a dark place. Erythrocytes were lysed for 15 min, and the
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9 135 debris was washed away. Before staining of the intracellular GR antibody and its
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12 136 isotype control (Bio-Rad AbD Serotec, Oxford, UK), surface-stained cells were fixed
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15 137 and permeabilized using the BD Transcription Factor Buffer Set (BD Pharmingen, San
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17 138 Diego, USA, Catalogue No. 562574). Monoclonal antibodies and their isotype controls
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20 139 were all purchased from BD Biosciences (San Jose, CA, USA). Details of all antibodies
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23 140 are shown in Supplemental Table 1. According to the manufacturer's recommendations,
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25 141 all antibodies and their isotype controls were used at a concentration of 1 μ L per 100
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27 142 μ L of whole blood. Samples were measured using the Gallios flow cytometer (Beckman
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30 143 Coulter, Brea, CA, USA) and analyzed using Gallios Software version 1.0 (Beckman
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33 144 Coulter). The flow cytometer was periodically calibrated by an engineer. Cells were
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35 145 stained for 20 min; thresholds were defined using the manufacturer's recommended
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38 146 isotype controls. Representative plots and gating strategy from a single sample are
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41 147 shown in Supplemental Figure 2. T cells were gated by CD3⁺CD4⁺ or CD3⁺CD8⁺, B
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44 148 cells were gated by CD3⁻CD19⁺, NK cells were gated by CD16⁺CD56⁺, and Tregs were
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47 149 gated by CD4⁺CD25^{high}CD127^{low}. At least 10,000 events were collected in the
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50 150 lymphocyte cell gate for each sample. Results are expressed as percentages and mean
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53 151 fluorescence intensity (MFI) values.

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56 152 Absolute CD3⁺ and CD4⁺ lymphocyte, NK cell, and Treg cell counts were obtained
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59 153 using Flow-Count fluorospheres (Beckman Coulter, Catalogue No. 7547053),
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154 according to the manufacturer's instructions. B, CD3⁺CD4⁺T, CD3⁺CD8⁺T, and Treg

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4 155 cell counts were calculated by their percentages in CD3⁺ or CD4⁺ lymphocytes
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6 156 multiplied by CD3⁺ or CD4⁺ lymphocyte counts.
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9 157 The secondary outcomes of this study were plasma total cortisol and ACTH levels
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11 158 after ROSC. Venous blood samples were collected in heparin anticoagulant tubes,
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14 159 centrifuged 10 min at 3000 rpm, and then stored at -80 °C. Plasma total cortisol
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17 160 (IMMULITE 2000 Cortisol, L2KCO2, UK) and ACTH (IMMULITE 2000 ACTH,
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19 161 L2KAC2, UK) levels were assayed using a chemiluminescent immunoassay on a
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22 162 Siemens automated analyzer (IMMULITE 2000 XPi; Siemens Healthcare Diagnostics,
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24 163 Erlangen, Germany). The equipment and reagents were calibrated by engineers before
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27 164 use. The lower detection limit of total cortisol was 2.00 ng/mL, and that of ACTH was
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30 165 5.00 pg/mL.
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32 166 **Sample size calculation and statistical analysis**

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35 167 The sample size was calculated using the PASS15.0 software (NCSS, LLC,
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37 168 Kaysville, UT, USA) and the non-parametric test method. The median GR expression
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40 169 was 0.93 and 0.80 in the healthy and CA groups, respectively, and the interquartile
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43 170 spacing was 0.1 and 0.3. According to the ratio of 1:2 between the two groups, with a
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46 171 test level of 0.05 and a confidence interval of 0.90, a total of 105 samples were required,
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48 172 comprising at least 35 in the healthy group and 70 in the CA group. The number of
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51 173 people included in the two groups in this study was 40 and 85, respectively, which met
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54 174 our research requirements. Data analysis was used in SPSS version 22.0 (IBM Corp.,
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56 175 Armonk, NY, USA). For normally distributed data, continuous variables are expressed
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59 176 as means with standard deviations. Since the data for total cortisol and ACTH levels
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4 177 had a skewed distribution, we compared our results with the natural logarithmic
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6 178 conversion values after adding 1 ($\ln [\text{total cortisol} + 1]$, $\ln [\text{ACTH} + 1]$). Measurement
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9 179 data with a skewed distribution are expressed as medians (25th and 75th percentiles).
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12 180 The Mann–Whitney U test was used to compare variables between groups. The
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14 181 qualitative parameters in the 2×2 contingency table were used for analysis. All
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17 182 statistical tests were two-tailed, and a P-value of <0.05 was considered statistically
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20 183 significant.

21 22 184 **Follow-up**

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25 185 Patients were classified into survivor and non-survivor groups according to the 28-
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27 186 day survival endpoint. Those with all-cause mortality within the follow-up period were
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30 187 considered non-survivors. If data were lost, the corresponding candidate was excluded.

31 32 33 188 **Patient and public involvement**

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35 189 Patients and/or the public were not involved in the design, or conduct, or reporting,
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38 190 or dissemination plans of this research.

39 40 41 42 43 192 **Results**

44 45 193 **Patient characteristics**

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48 194 40 healthy individuals and 85 patients who experienced CA were analyzed. The
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50 195 demographics and clinical characteristics of both groups are shown in Table 1. In this
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53 196 study, acute cardiac and brain events were the main causes of CA, with those in the
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56 197 latter category emanating from strokes. Other causes of CA included poisoning
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59 198 (including carbon monoxide poisoning) and hypokalemia. Sex and age were not
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199 significantly different between the CA and healthy control groups. The comparisons of
 200 clinical characteristics of the survivor and non-survivor groups based on 28-day
 201 survival are shown in Supplemental Table 2. The APACHE II and SOFA scores were
 202 significantly different between the CA and healthy control groups ($P<0.001$ for all) and
 203 survivor and non-survivor groups ($P<0.001$ and $P=0.011$, respectively).

204

205 **Table 1.** Patient Characteristics at Admission

Characteristics	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)
Age (years), median [IQR]	64.0 (54.3, 69.8)	65.0 (55.0, 74.0)
Male/Female (n)	23/17	58/27
Previous medical history, n (%)		
Hypertension	5 (12.5%)	38 (44.7%)
Diabetes	3 (7.5%)	27 (31.8%)
Coronary heart disease	2 (5.0%)	29 (34.1%)
Chronic lung disease	1 (2.5%)	9 (10.6%)
Chronic kidney disease	0	9 (10.6%)
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)
White cell count ($\times 10^9/L$), median [IQR]	5.81 (4.85, 6.53)	13.56 (10.84, 18.29)
APACHE II score, mean \pm SD	0	32.9 \pm 6.5
SOFA score, median [IQR]	0	11.5 (8.5, 14.0)
28-day mortality, n (%)		65 (76.5%)
28-day CPC 1–2, n (%)		14 (16.5%)

206 Abbreviations: IQR: interquartile range; ROSC: return of spontaneous circulation;
 207 VF: ventricular fibrillation; VT: ventricular tachycardia; MAP: mean arterial pressure;
 208 APACHE II: acute physiology and chronic health evaluation; SOFA: sequential
 209 organ failure assessment; SD: standard deviation; CPC: cerebral performance
 210 category.

211 **Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts after** 212 **ROSC**

213 The T and B lymphocyte, NK cell, and Treg cell counts were significantly lower after
 214 ROSC in patients who experienced CA than in healthy controls ($P < 0.001$ for all).
 215 Additionally, the $CD3^+CD4^+/T$ lymphocyte, $CD3^+CD8^+/T$ lymphocyte, and Treg
 216 cell/ $CD4^+$ T lymphocyte ratios were significantly lower after ROSC in patients who
 217 experienced CA than in healthy controls ($P < 0.001$ for all) (Fig. 1; Supplemental Table
 218 3). However, there were no significant differences in these cell counts and ratios
 219 between survivors ($n=20$) and non-survivors ($n=65$) ($P > 0.05$ for all) (Supplemental
 220 Table 4).

221 **GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells after** 222 **ROSC**

223 The MFI and percentages of GR expression in B and T lymphocytes, NK cells, and

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4 224 CD3⁺CD8⁺ T lymphocytes were significantly lower after ROSC in patients who
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6 225 experienced CA than in healthy individuals (P<0.01 for all) (Fig. 2A–D, G, H, K, L).
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9 226 There were also significant reductions in the MFI in Treg cells and CD3⁺CD4⁺ T
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11 227 lymphocytes (P<0.05 for all) (Fig. 2E, I) but not in the percentages of GR expression
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14 228 (P>0.05 for all) (Fig. 2F, J; Supplemental Table 5). However, there were no significant
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17 229 differences in the MFI and percentages of GR expression in these cells between
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20 230 survivors and non-survivors (P>0.05 for all) (Supplemental Table 6).

231 **Changes in plasma total cortisol and ACTH levels after ROSC**

232 We measured the plasma total cortisol and ACTH levels of the 40 healthy individuals
233 and 85 patients who experienced CA (two samples were excluded because their total
234 cortisol levels were not measured). Plasma total cortisol levels were significantly higher
235 in patients who experienced CA than in healthy controls (P<0.001), but ACTH levels
236 were not (Fig. 3A, C). No significant differences in ln (total cortisol+1) and ln
237 (ACTH+1) values were observed between survivors and non-survivors (P>0.05 for all)
238 (Fig. 3B, D).

240 **Discussion**

241 In this study, we examined the levels of GR expression and plasma corticosteroids
242 in patients with CA in the early period after ROSC. We found that GR expression in
243 circulatory T and B lymphocytes, NK cells, and Treg cells, cell counts and ratios in
244 patients with CA was significantly lower compared to that in controls. Furthermore,
245 plasma total cortisol levels in patients with CA were significantly higher compared to

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4 246 the controls.

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6 247 The ischemia-reperfusion response initiates an acute inflammatory response that
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9 248 contributes to post-resuscitation shock after CA.[27] The immune response of patients
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12 249 who experience CA is impaired, and the systemic inflammatory response increases. [6,
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14 250 28] The T and B lymphocyte, NK cell, and Treg cell counts and CD3⁺CD4⁺/T,
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17 251 CD3⁺CD8⁺/T, and Treg cell/CD4⁺ T lymphocyte ratios were significantly reduced after
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20 252 ROSC. NK cells, which are special innate immune cells with cytotoxic functions
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23 253 similar to CD3⁺CD8⁺ T lymphocytes, mainly distinguish infected and stressed cells
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26 254 from healthy cells and eliminate intracellular infection and dysfunctional cells. [29, 30]
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29 255 T lymphocytes are also crucial because they function as adaptive immune cells to
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32 256 control and eliminate the infection. [29] Moreover, B and T lymphocytes mediate
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35 257 humoral and cellular immunity, respectively. This study was performed earlier and
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38 258 involved a more comprehensive assessment of the immune system of patients who
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41 259 experienced CA. Our findings more substantially supported the rapid emergence of
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44 260 immune dysfunction in these patients after ROSC than in previous reports.

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46 261 The binding of GCs to GR inside different peripheral blood mononuclear cells
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49 262 (PBMC) leads to changes in the ability of cells to regulate apoptosis, proliferation, and
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52 263 activity, and GC-GR complexes limit the transcription (trans-repression) of
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55 264 inflammatory genes, including those encoding for proinflammatory cytokines.[31, 32]
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58 265 This study is the first to explore GR expression in circulating immune cells in patients
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61 266 who experienced CA after ROSC. We observed that GR expression in B and T
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64 267 lymphocytes, NK cells, and CD3⁺CD8⁺ T lymphocytes decreased significantly in

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4 268 patients who experienced CA, whereas the percentage of GR⁺ Treg cells and
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6 269 CD3⁺CD4⁺ T lymphocytes decreased slightly. Moreover, we observed a more
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9 270 significant decrease in the MFI of GR expression in Treg cells and CD3⁺CD4⁺ T
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11 271 lymphocytes but not in the percentage of GR expression. Previous studies have found
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14 272 decreased expression of GRs in peripheral polymorphonuclear cells in critically ill
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17 273 patients, [21] and antagonism to GRs aggravates viral and bacterial infections. [33]
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19 274 GCs induced upon infections help to maintain homeostasis and mitigate the life-
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22 275 threatening impact of sepsis on the host.[31] Although studies have reported that the
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25 276 use of GCs during and after CPR seems to confer benefits concerning ROSC rates and
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28 277 long-term survival, the evidence is scant. [13,18,34,35] Since cortisol signaling is
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31 278 mediated by GRs, we hypothesized that the differential responses of CA patients to GC
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34 279 may be related to their levels of GR expression. This study suggests that the decrease
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37 280 in intracellular GR expression in patients who experienced CA is one of the causes of
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40 281 GC resistance due to insufficient binding of GRs and GCs, GC insensitivity, and the
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43 282 inability of GCs to exert anti-inflammatory and immunosuppressive effects effectively.
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46 283 These findings may also explain why different results regarding the clinical application
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49 284 of GCs have been reported previously. Furthermore, it is vital to measure GR levels as
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52 285 sufficient expression of GR is essential for mediating adequate GC effects during and
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55 286 after CPR.

53 287 We also found that the total plasma cortisol levels were significantly higher in
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56 288 patients who experienced CA, but ACTH levels were not. High levels of inflammatory
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59 289 cytokines inhibit ACTH release. [18] During critical illness, the body does not
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4 290 sufficiently metabolize cortisol. [36] In addition, the continuous increase in plasma
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6 291 cortisol levels may trigger the negative feedback pathway of the hypothalamic-
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9 292 pituitary-adrenal axis, inhibiting the release of ACTH and cortisol and eventually
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11 293 leading to adrenal insufficiency [37]. These factors may explain the opposite trends of
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14 294 plasma ACTH and cortisol levels in the patients included in this study and who
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17 295 experienced CA. Notably, this result suggests that low GR expression levels are not
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20 296 matched by high plasma total cortisol levels in patients who experienced CA. The
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22 297 dissociation between low GR expression and high cortisol implies an abnormal stress
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25 298 response. [38] Previous studies have reported that GR-action was clearly suppressed
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27 299 throughout critical illness; GR resistance could not be overcome by further increasing
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30 300 glucocorticoid availability.[21,39,40] Adequate GR levels and function are also
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32 301 required for normal GC function, which may explain differences in the responsiveness
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35 302 of cardiac arrest patients to exogenous steroid administration or endogenous cortisol
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38 303 secretion. Thus, actual GR levels cannot be reflected by measuring total cortisol levels
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41 304 alone. Therefore, the GR level should be considered when applying personalized GC
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44 305 therapy. The determination of GR expression might help to screen those who might
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46 306 respond better to glucocorticoid prescription.

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50 308 **Limitations**

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53 309 Our study has several limitations. First, to assess changes, we only enrolled patients
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56 310 who experienced CA and had signs of systemic ischemic hypoxia, such as GCS <8 after
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59 311 ROSC. The patients were not stratified by age, sex, the occurrence of comorbidities, or

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4 312 mild systemic ischemic hypoxia. Second, since this was a preliminary observational
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6 313 study, we observed only early changes. A more relevant control group and dynamic
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9 314 observations obtained over a longer duration would be helpful to understand the
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11 315 significance of GR expression in evolving immunity during the clinical course of CA
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14 316 after ROSC. Third, the samples used in this study were from clinical laboratories; thus,
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17 317 plasma total cortisol and ACTH in the samples were at risk of degradation before we
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19 318 collected the samples. Finally, we did not discuss the changes in and roles of GR
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21 319 isoforms, free cortisol, and corticosteroid-binding globulin. Therefore, future studies
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24 320 on these aspects are warranted to better understand the immunosuppressive effects of
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27 321 ROSC among patients who experienced CA.

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30 322 In conclusion, this study revealed that GR expression, cell counts and ratios rapidly
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32 323 decreased, whereas plasma total cortisol levels increased, in the early period after
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35 324 ROSC among CA patients. These findings may provide important information about
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38 325 GR expression levels and function, and immunosuppressive status in these patients.

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44
45 328 this study and colleagues from the emergency department who provided support. And
46
47
48 329 we are grateful for the efforts of the staff for ongoing resuscitation in hospitals.

49
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52
53 331 YNY searched the literature and contributed to the experimental studies, data analysis,
54
55
56 332 and manuscript writing. ZRT, CCH, and LA collected and analyzed data. JBL and MRX

333 helped with the statistical analyses. All authors have read and approved the final
334 manuscript.

335 **Competing interests:** All authors declare no competing interest associated with this
336 project.

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338 commercial or not-for-profit sectors.

339 **Provenance and peer review:** Not commissioned; externally peer-reviewed.

340 **Data sharing statement:** All data relevant to the study are included in the article or
341 uploaded as supplementary information. Due to privacy and ethical concerns, data can
342 not be shared.

344 **Ethics statements**

345 **Patient consent for publication:** Not applicable.

346 **Ethics approval:** This study was approved by the Medical Ethics Committee of Beijing
347 Chaoyang Hospital (2013-KE-1). Because CA is sudden and life-threatening, the
348 consent was usually obtained orally from relatives or bystanders and in writing with
349 some delay from relatives or bystanders after successful resuscitation.

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351 **References**

- 352 [1] Myat A, Song KJ, Rea T. Out-of-hospital cardiac arrest: current concepts. *Lancet*,
353 Mar 10, 2018. DOI: 10.1016/S0140-6736(18)30472-0.
- 354 [2] Zhang S. Sudden cardiac death in China: current status and future perspectives.
355 *Europace*, Oct, 2015. DOI: 10.1093/europace/euv143.
- 356 [3] Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology,
357 pathophysiology, treatment, and prognostication. A Scientific Statement from the
358 international Liaison Committee on Resuscitation; the American Heart Association
359 Emergency cardiovascular Care Committee; the Council on Cardiovascular Surgery
360 and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the
361 Council on Clinical Cardiology; the Council on Stroke. *Resuscitation*, Dec, 2008. DOI:
362 10.1016/j.resuscitation.2008.09.017.
- 363 [4] Su CP, Wu JH, Yang MC, et al. Demographics and clinical features of
364 postresuscitation comorbidities in long-term survivors of out-of-hospital cardiac arrest:
365 A national follow-up study. *Biomed Res Int*, 2017. DOI: 10.1155/2017/9259182.
- 366 [5] Tsai MS, Chiang WC, Lee CC, et al. Infections in the survivors of out-of-hospital
367 cardiac arrest in the first 7 days. *Intensive Care Med*, May 31, 2005. DOI:
368 10.1007/s00134-005-2612-6.
- 369 [6] Adrie C, Adib-Conquy M, Laurent I, et al. Successful cardiopulmonary
370 resuscitation after cardiac arrest as a "sepsis-like" syndrome. *Circulation*, Jul 30, 2002.
371 DOI: 10.1161/01.cir.0000023891.80661.ad.
- 372 [7] Hall ED. Neuroprotective actions of glucocorticoid and nonglucocorticoid steroids

-
- 1
2
3
4 373 in acute neuronal injury. *Cell Mol Neurobiol*, Aug 13, 1993. DOI:
5
6 374 10.1007/BF00711581.
7
8
9 375 [8] de Jong MF, Beishuizen A, de Jong MJ et al. The pituitary-adrenal axis is activated
10
11 376 more in non-survivors than in survivors of cardiac arrest, irrespective of therapeutic
12
13 377 hypothermia. *Resuscitation*, Sep, 2008. DOI: 10.1016/j.resuscitation.2008.03.227.
14
15
16 378 [9] Mosaddegh R, Kianmehr N, Mahshidfar B et al. Serum cortisol level and adrenal
17
18 379 reserve as a predictor of patients' outcome after successful cardiopulmonary
19
20 380 resuscitation. *J Cardiovasc Thorac Res*, 2016. DOI: 10.15171/jcvtr.2016.12.
21
22
23 381 [10] Hékimian G, Baugnon T, Thuong M, et al. Cortisol levels and adrenal reserve after
24
25 382 successful cardiac arrest resuscitation. *Shock*, Aug, 2004. DOI:
26
27 383 10.1097/01.shk.0000132489.79498.c7.
28
29
30 384 [11] Tavakoli N, Bidari A, Shams Vahdati S. Serum Cortisol levels as a predictor of
31
32 385 neurologic survival in successfully resuscitated victims of cardiopulmonary arrest. *J*
33
34 386 *Cardiovasc Thorac Res*, 2012. DOI: 10.5681/jcvtr.2012.026.
35
36
37 387 [12] Soar J, Callaway CW, Aibiki M, et al. Resuscitation- Part 4: advanced life support:
38
39 388 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency
40
41 389 Cardiovascular Care Science with Treatment Recommendations. *Resuscitation*, Oct,
42
43 390 2015. DOI: 10.1016/j.resuscitation.2015.07.042.
44
45
46 391 [13] Mentzelopoulos SD, Malachias S, Chamos C, et al. Vasopressin, steroids, and
47
48 392 epinephrine and neurologically favorable survival after in-hospital cardiac arrest: a
49
50 393 randomized clinical trial. *JAMA*, Jul 17, 2013. DOI: 10.1001/jama.2013.7832.
51
52
53 394 [14] Tsai MS, Chuang PY, Yu PH, et al. Glucocorticoid use during cardiopulmonary
54
55
56
57
58
59
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4
5
6
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10
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45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

-
- 395 resuscitation may be beneficial for cardiac arrest. *Int J Cardiol*, Nov 1, 2016. DOI:
396 10.1016/j.ijcard.2016.08.017.
- 397 [15] Niimura T, Zamami Y, Koyama T, et al. Hydrocortisone administration was
398 associated with improved survival in Japanese patients with cardiac arrest. *Sci Rep*,
399 Dec 20, 2017. DOI: 10.1038/s41598-017-17686-3.
- 400 [16] Chalkias A, Xanthos T. Post-cardiac arrest syndrome: mechanisms and evaluation
401 of adrenal insufficiency. *World J Crit Care Med*, Feb 4, 2012. DOI:
402 10.5492/wjccm.v1.i1.4.
- 403 [17] Buddineni JP, Callaway C, Huang DT. Epinephrine, vasopressin and steroids for
404 in-hospital cardiac arrest: the right cocktail therapy? *Crit Care*, Jun 2, 2014. DOI:
405 10.1186/cc13903.
- 406 [18] Varvarousi G, Stefaniotou A, Varvaroussis D et al. Glucocorticoids as an emerging
407 pharmacologic agent for cardiopulmonary resuscitation. *Cardiovasc Drugs Ther*, Oct,
408 2014. DOI: 10.1007/s10557-014-6547-4.
- 409 [19] Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease.
410 *Trends Pharmacol Sci*, Sep, 2013. DOI: 10.1016/j.tips.2013.07.003.
- 411 [20] Norbiato G, Bevilacqua M, Vago T, et al. Cortisol resistance in acquired
412 immunodeficiency syndrome. *J Clin Endocrinol Metab*, Mar, 1992. DOI:
413 10.1210/jcem.74.3.1740494.
- 414 [21] Vassiliou AG, Floros G, Jahaj E, et al. Decreased glucocorticoid receptor
415 expression during critical illness. *Eur J Clin Invest*, Apr, 2019. DOI: 10.1111/eci.13073.
- 416 [22] Alder MN, Opoka AM, Wong HR. The glucocorticoid receptor and cortisol levels

-
- 1
2
3
4 417 in pediatric septic shock. *Crit Care*, Sep 29, 2018. DOI: 10.1186/s13054-018-2177-8.
5
6
7 418 [23] Qi Z, Liu Q, Zhang Q et al. Overexpression of programmed cell death-1 and human
8
9 419 leucocyte antigen-DR on circulatory regulatory T cells in out-of-hospital cardiac arrest
10
11 420 patients in the early period after return of spontaneous circulation. *Resuscitation*, Sep,
12
13 421 2018. DOI: 10.1016/j.resuscitation.2018.06.023.
14
15
16 422 [24] Qi Z, An L, Liu B, et al. Patients with out-of-hospital cardiac arrest show decreased
17
18 423 human leucocyte antigen-DR expression on monocytes and B and T lymphocytes after
19
20 424 return of spontaneous circulation. *Scand J Immunol*, Oct, 2018. DOI:
21
22 425 10.1111/sji.12707.
23
24
25 426 [25] Perkins GD, Travers AH, Berg RA, et al. Resuscitation-Part 3: adult basic life
26
27 427 support and automated external defibrillation: 2015 International Consensus on
28
29 428 Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with
30
31 429 Treatment Recommendations. *Resuscitation*, Oct, 2015. DOI:
32
33 430 10.1016/j.resuscitation.2015.07.041.
34
35
36 431 [26] Jacobs I, Nadkarni V, Bahr J, et al. Cardiac arrest and cardiopulmonary
37
38 432 resuscitation outcome reports: update and simplification of the Utstein templates for
39
40 433 resuscitation registries. A statement for healthcare professionals from a task force of
41
42 434 the international liaison committee on resuscitation (American Heart Association,
43
44 435 European Resuscitation Council, Australian Resuscitation Council, New Zealand
45
46 436 Resuscitation Council, Heart and Stroke Foundation of Canada, InterAmerican Heart
47
48 437 Foundation, Resuscitation Council of Southern Africa). *Resuscitation*, Dec, 2004. DOI:
49
50 438 10.1016/j.resuscitation.2004.09.008.
51
52
53
54
55
56
57
58
59
60

-
- 1
2
3
4 439 [27] Lindner KH, Strohmenger HU, Ensinger H, Hetzel WD, Ahnefeld FW, Georgieff
5
6 440 M. Stress hormone response during and after cardiopulmonary resuscitation.
7
8
9 441 Anesthesiology, Oct, 1992. DOI: 10.1097/00000542-199210000-00008.
10
11
12 442 [28] Beurskens CJ, Horn J, de Boer AM, et al. Cardiac arrest patients have an impaired
13
14 443 immune response, which is not influenced by induced hypothermia. Crit Care, Jul 30,
15
16 444 2014. DOI: 10.1186/cc14002.
17
18
19 445 [29] Lanier LL. NK cell recognition. Annu Rev Immunol, 2005. DOI:
20
21 446 10.1146/annurev.immunol.23.021704.115526.
22
23
24 447 [30] Vivier E, Tomasello E, Baratin M et al. Functions of natural killer cells. Nat
25
26 448 Immunol, May, 2008. DOI: 10.1038/ni1582.
27
28
29 449 [31] Zen M, Canova M, Campana C, et al. The kaleidoscope of glucocorticoid effects on
30
31 450 immune system. Autoimmun Rev, Apr, 2011. DOI: 10.1016/j.autrev.2010.11.009.
32
33
34 451 [32] Vandewalle J, Libert C. Glucocorticoids in Sepsis: To be or not to be. Front
35
36 452 Immunol, Jul 21, 2020. DOI: 10.3389/fimmu.2020.01318.
37
38
39 453 [33] Webster JI, Sternberg EM. Role of the hypothalamic-pituitary-adrenal axis,
40
41 454 glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial
42
43 455 and viral products. J Endocrinol, May, 2004. DOI: 10.1677/joe.0.1810207.
44
45
46 456 [34] Andersen LW, Isbye D, Kjærgaard J, et al. Effect of Vasopressin and
47
48 457 methylprednisolone vs placebo on return of spontaneous circulation in patients with In-
49
50 458 hospital cardiac arrest: A randomized clinical trial. JAMA, Oct 26, 2021. DOI:
51
52 459 10.1001/jama.2021.16628.
53
54
55 460 [35] Smithline H, Rivers E, Appleton T, Nowak R. Corticosteroid supplementation
56
57
58
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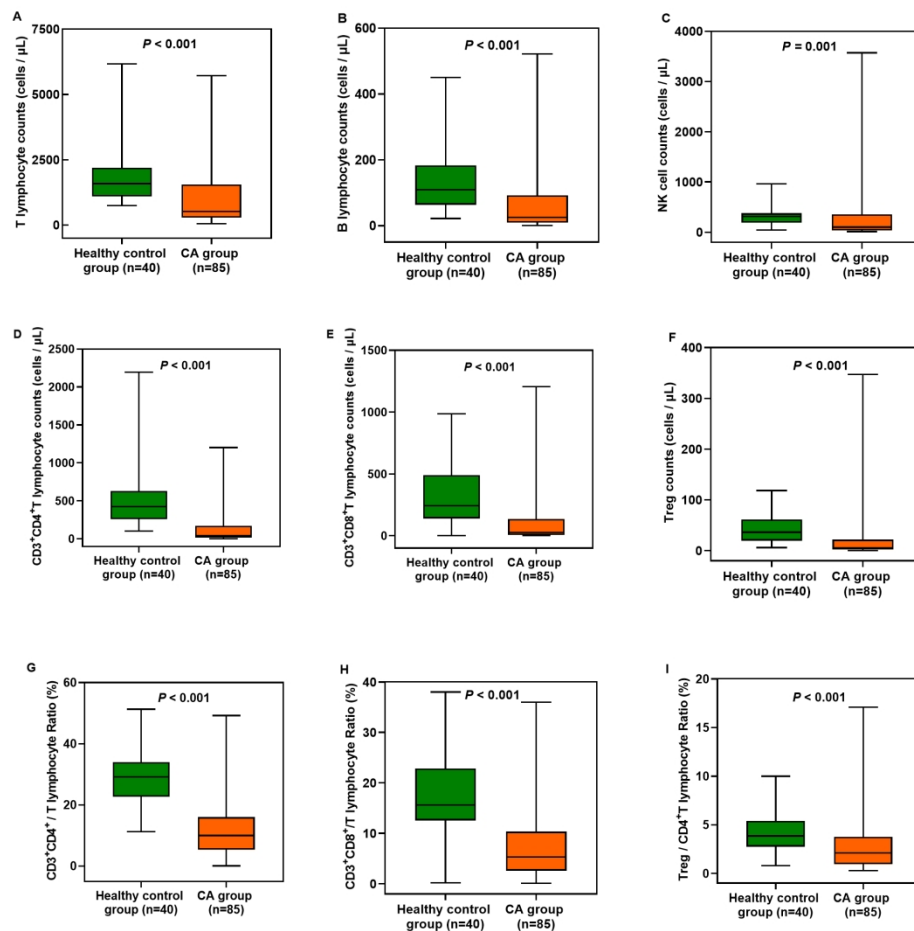
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4 461 during cardiac arrest in rats. *Resuscitation*, Jun, 1993. DOI: 10.1016/0300-
5
6 462 9572(93)90123-8.
7
8
9 463 [36] Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during
10
11 464 critical illness. *N Engl J Med*, Apr 18, 2013. DOI: 10.1056/NEJMoa1214969.
12
13
14 465 [37] Peeters B, Langouche L, Van den Berghe G. Adrenocortical stress response during
15
16 466 the course of critical illness. *Compr Physiol*, Dec 12, 2017. DOI:
17
18 467 10.1002/cphy.c170022.
19
20
21 468 [38] Vassiliou AG, Stamogiannos G, Jahaj E, et al. Longitudinal evaluation of
22
23 469 glucocorticoid receptor alpha/beta expression and signaling, adrenocortical function
24
25 470 and cytokines in critically ill steroid-free patients. *Mol Cell Endocrinol*, Feb 5, 2020.
26
27 471 DOI: 10.1016/j.mce.2019.110656.
28
29
30 472 [39] Indyk JA, Candido-Vitto C, Wolf IM, et al. Reduced glucocorticoid receptor
31
32 473 protein expression in children with critical illness. *Horm Res Paediatr. Horm Res*
33
34 474 *Paediatr*. 2013. DOI: 10.1159/000348290.
35
36
37 475 [40] Téblick A, Van Dyck L, Van Aerde N, et al. Impact of duration of critical illness
38
39 476 and level of systemic glucocorticoid availability on tissue-specific glucocorticoid
40
41 477 receptor expression and actions: A prospective, observational, cross-sectional human
42
43 478 and two translational mouse studies. *EBioMedicine*. 2022. DOI:
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45 479 10.1016/j.ebiom.2022.104057
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4 480 **Figure legends**

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6 481 **Fig. 1.** Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts,
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9 482 CD3⁺CD4⁺/T, CD3⁺CD8⁺/T, and Treg/CD4⁺T lymphocyte ratios between the healthy
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11 483 control group and CA group. The CA group showed significant differences compared
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14 484 with the healthy control group (P<0.001). CA, cardiac arrest; CD, cluster-of-
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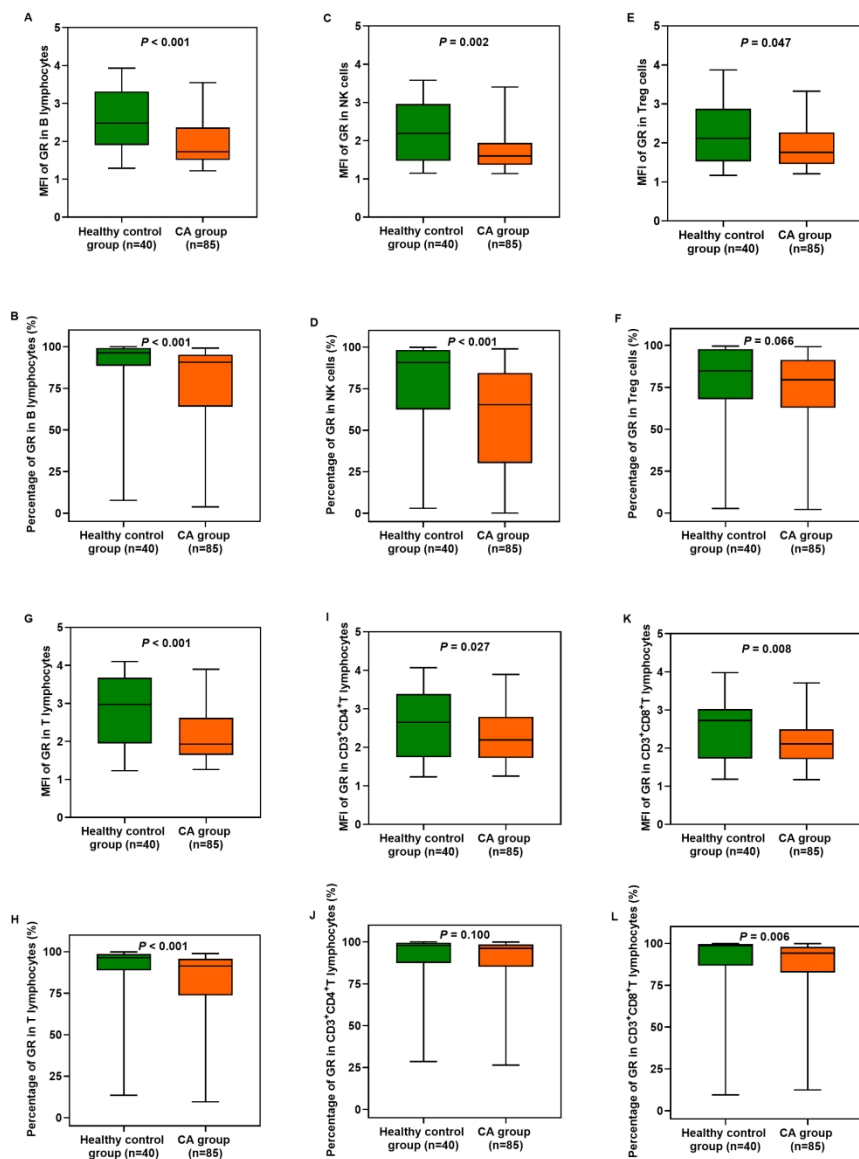
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19 486 **Fig. 2.** Expression of GRs in circulatory T and B lymphocytes, NK cells, and Treg cells
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22 487 in the healthy control group and CA group. The CA group showed significant
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25 488 differences compared with the healthy control group (P<0.05). CA, cardiac arrest; CD,
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28 489 cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC,
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30 490 return of spontaneous circulation; Treg, regulatory T.

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32 491 **Fig. 3.** (A, B) Plasma total cortisol and ACTH levels (the natural logarithmic
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35 492 conversion values after adding 1) after ROSC in the healthy control group and CA
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38 493 group. (C, D) Plasma total cortisol and ACTH levels in survivors and non-survivors
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41 494 after ROSC. The CA group showed significant differences compared with the healthy
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44 495 control group (P<0.05). ACTH, adrenocorticotrophic hormone; CA, cardiac arrest;
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46 496 ROSC, return of spontaneous circulation.



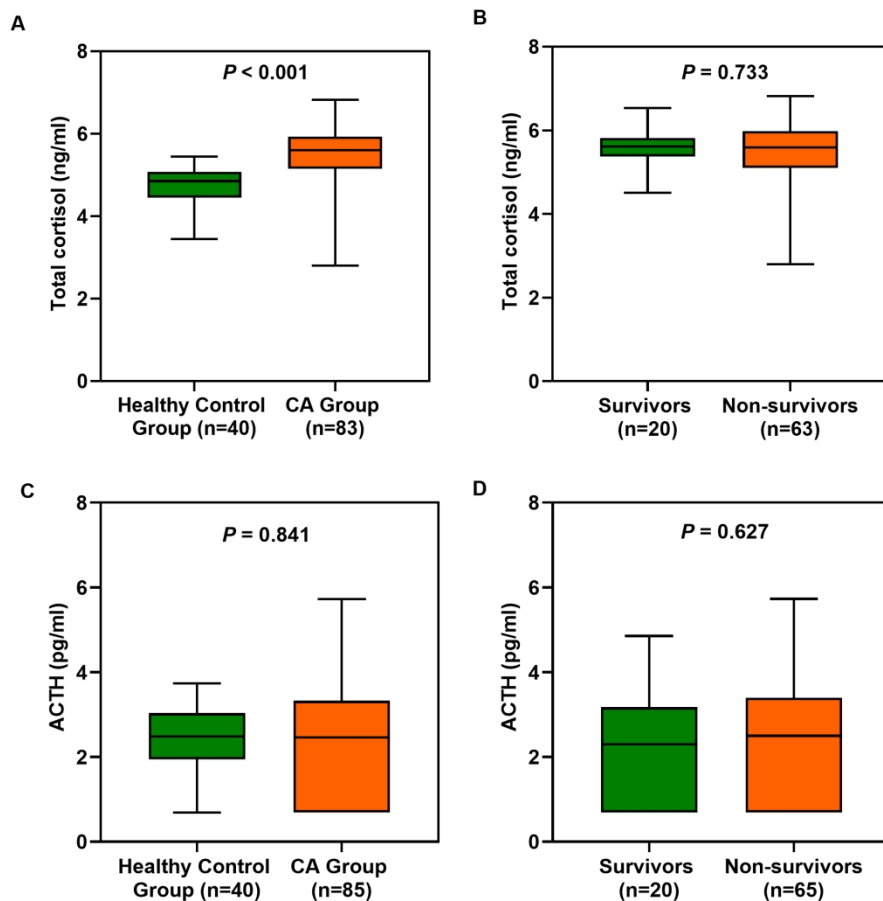
Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and CD3+CD4+/T, CD3+CD8+/T, and Treg/CD4+T lymphocyte ratios between the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group ($P < 0.001$). CA, cardiac arrest;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

187x183mm (300 x 300 DPI)



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; CD, cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC, return of spontaneous circulation; Treg, regulatory T.

199x256mm (300 x 300 DPI)



(A, B) Plasma total cortisol and ACTH levels (the natural logarithmic conversion values after adding 1) after ROSC in the healthy control group and CA group. (C, D) 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.

185x178mm (300 x 300 DPI)

Electronic supplemental material**Glucocorticoid receptor expression in patients with cardiac arrest in the early period after the return of spontaneous circulation: A prospective observational single-center study**

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Contents

Supplemental Figure 1

Supplemental Figure 2

Supplemental Table 1

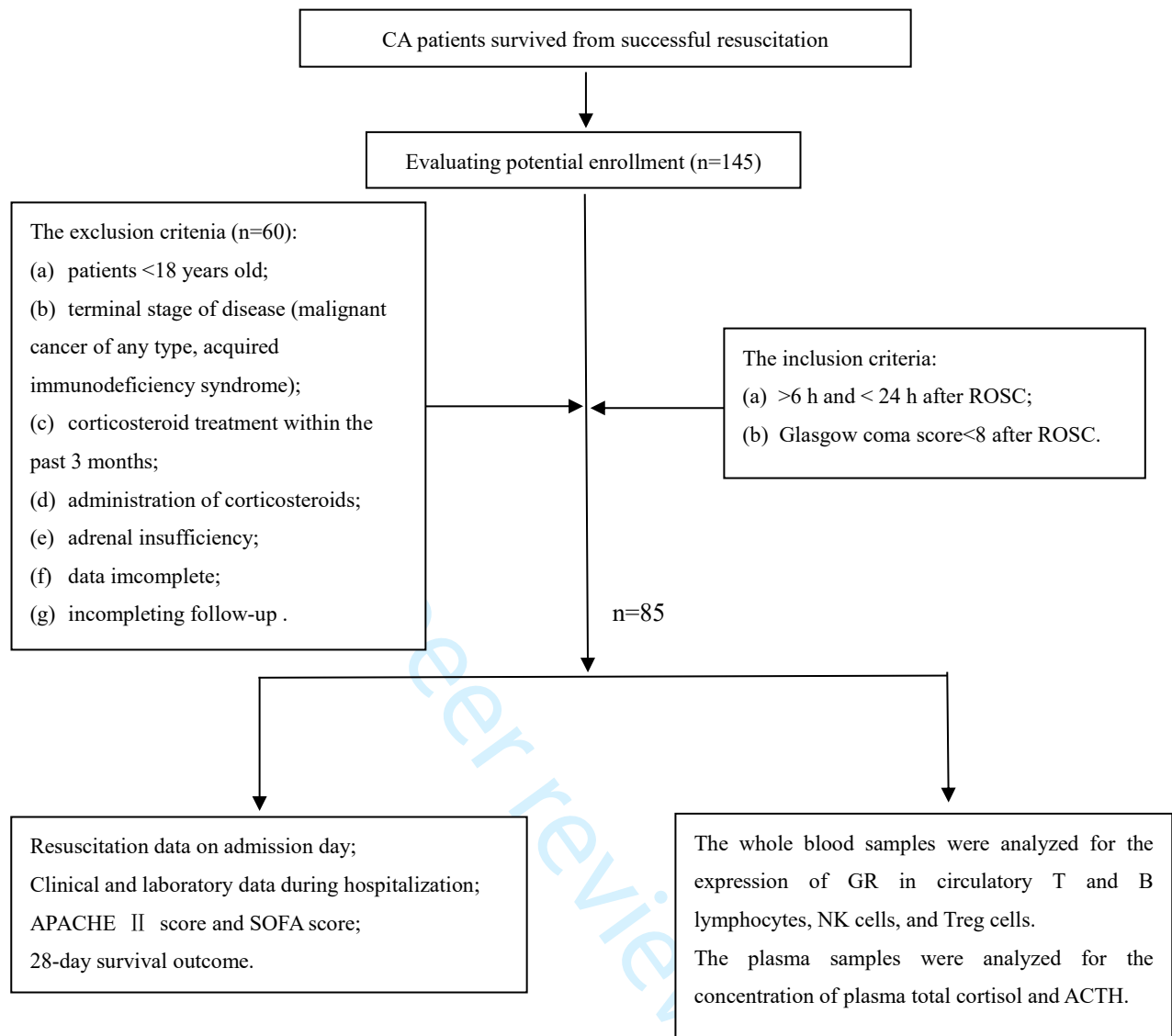
Supplemental Table 2

Supplemental Table 3

Supplemental Table 4

Supplemental Table 5

Supplemental Table 6



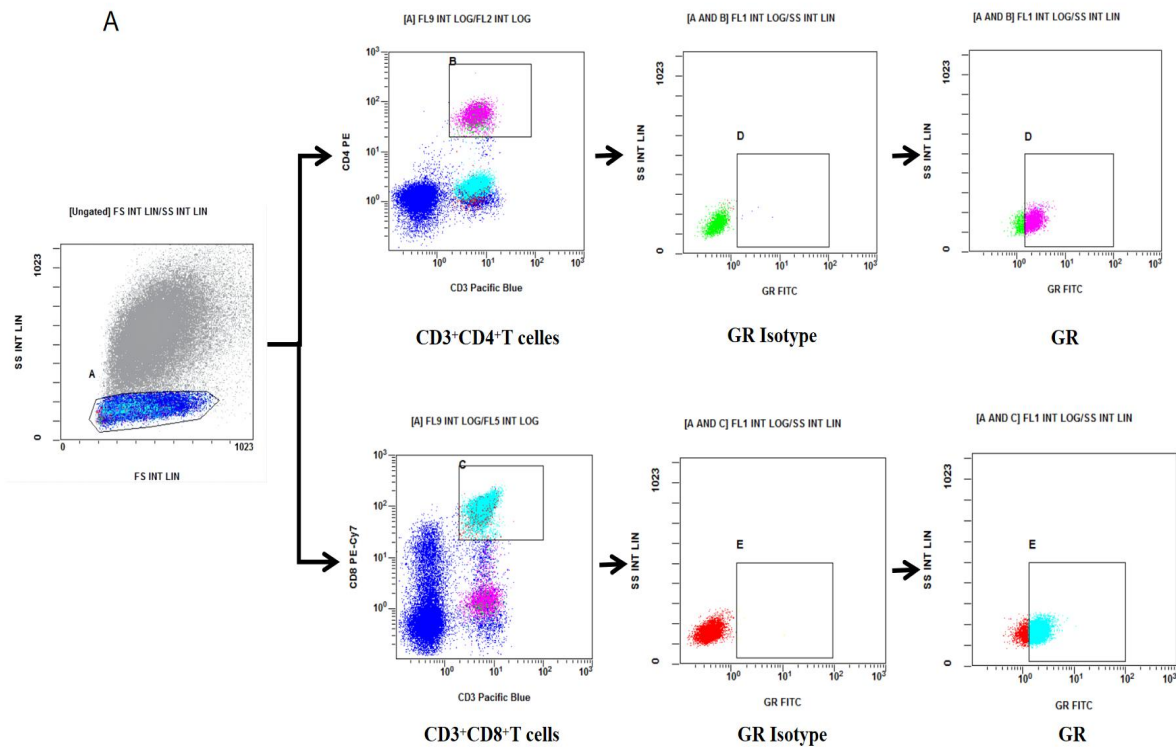
Supplemental Figure 1. The flow chart of the study.

Abbreviations: CA, cardiac arrest; ROSC, return of spontaneous circulation; APACHE II, acute physiology and chronic health evaluation II; SOFA, sequential organ failure assessment; GR, glucocorticoid receptor; Treg, regulatory T; ACTH, adrenocorticotrophic hormone.

Supplemental Figure 2. Representative plots and gating strategies for analyzing glucocorticoid receptor (GR) in the whole blood.

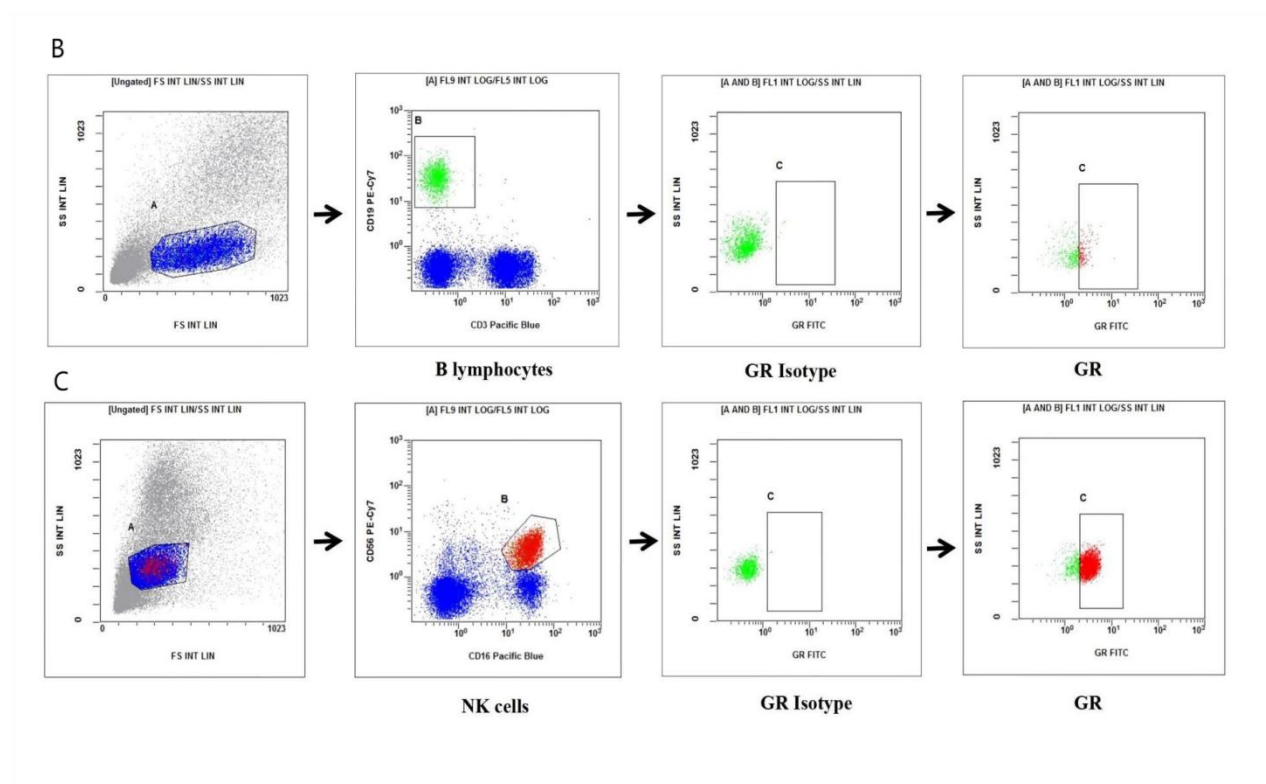
GR expression levels were determined on T cells, B cells, NK cells, and T regulatory (Treg) cells. Single cells were gated from all cellular events (FSC/SSC gate). B cells were identified as CD3⁻CD19⁺ cells. NK cells were identified as CD16⁺56⁺ cells. T cells were identified as CD3⁺CD4⁺ T cells and CD3⁺CD8⁺ T cells. Treg cells were identified as CD4⁺CD25^{high}CD127^{low}.

A. Expression of GR on T cells

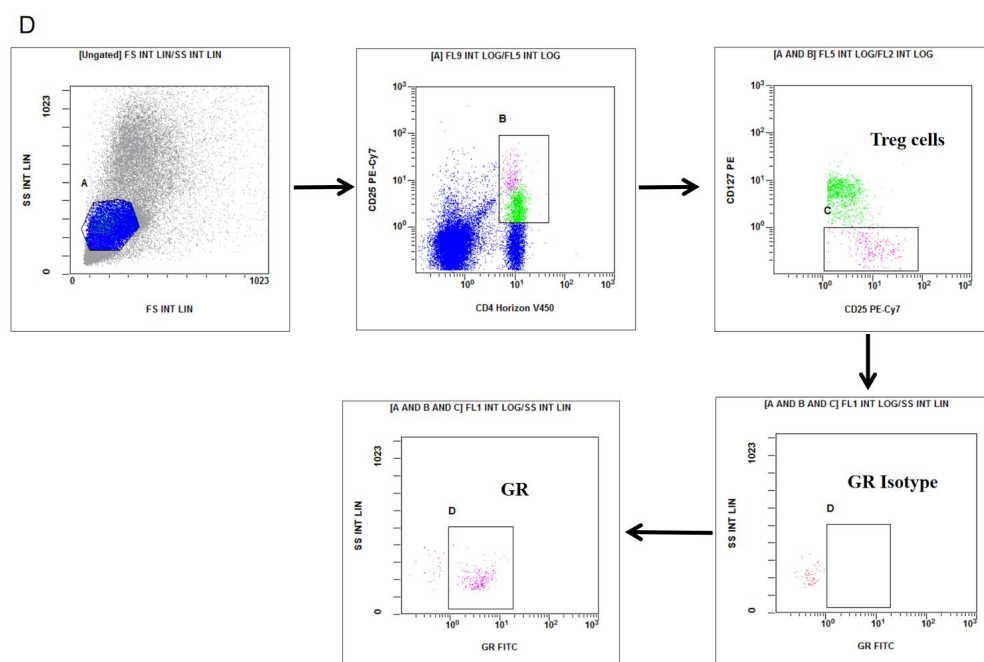


B. Expression of GR on B cells

C. Expression of GR on NK cells



D. Expression of GR on Treg cells



Supplemental Table 1. Details of antibodies for flow cytometry.

Antigen	Catalog Number	Fluorescein Conjugate	Source
CD3	558117	Pacific Blue	BD Pharmingen ^a
CD4	555347	PE	BD Pharmingen
CD4	560345	Horizon V450	BD Pharmingen
CD8	557746	PE-Cy7	BD Pharmingen
CD19	557835	PE-Cy7	BD Pharmingen
CD16	558122	Pacific Blue	BD Pharmingen
CD56	557747	PE-Cy7	BD Pharmingen
CD25	557741	PE-Cy7	BD Pharmingen
CD127	557938	PE	BD Pharmingen
GR	MCA2469F	FITC	Bio-Rad ^b
Mouse IgG1 Isotype	MCA928F	FITC	Bio-Rad
Mouse IgG1, κ Isotype	557872	PE-Cy7	BD Pharmingen
Mouse IgG1, κ Isotype	554680	PE	BD Pharmingen
Mouse IgG1, κ Isotype	558120	Pacific Blue	BD Pharmingen

^a BD Pharmingen, San Diego, USA; ^b Bio-Rad AbD Serotec, Oxford, UK.

Abbreviations: CD, cluster-of-differentiation; PE, phycoerythrin; FITC, fluorescein isothiocyanate; GR, glucocorticoid receptor; Ig: immunoglobulin.

Supplemental Table 2. Characteristics of CA survivors and non-survivors on admission.

	Survivors (n=20)	Non-survivors (n=65)
Age (years), median [IQR]	59.0 (53.3, 72.8)	66.0 (59.0, 75.5)
Male/Female (n)	12/8	46/19
Cardiac arrest cause (n, %)		
Cardiac	10 (50.0%)	24 (36.9%)
Non-Cardiac	10 (50.0%)	41 (63.1%)
Initial resuscitation		
Time to ROSC (min), median [IQR]	15.0 (7.3, 26.0)	20.0 (15.0, 30.0)
Adrenaline (mg), median [IQR]	1.0 (0.0, 3.0)	2.0 (0.0, 5.0)
Initial rhythm VF/VT, n (%)	11 (55.0%)	19 (29.2%)
MAP (mmHg), median [IQR]	89.9 (70.5, 104.9)	70.7 (50.0, 93.5)
White cell count ($\times 10^9/L$), median [IQR]	12.40 (6.98, 18.76)	13.80 (11.67, 18.20)
Lactate (mmol/L), median [IQR]	3.50 (1.33, 7.05)	7.50 (3.80, 11.20)
APACHE II score, mean \pm SD	27.8 \pm 6.6	34.4 \pm 5.6
SOFA score, median [IQR]	9.0 (7.3, 11.8)	12.0 (9.0, 15.0)

Data are presented as mean \pm SD or interquartile range (IQR) as appropriate. 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.

Supplemental Table 3. The flow cytometry results of cell counts and ratios of the healthy control group and successful resuscitation group

	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)	Z-value	P-value
T lymphocyte count (cells / μ L)	1586.0 (1101.5, 2192.5)	514.0 (287.5, 1555.0)	-4.515	<0.001
NK cell count (/ μ L)	311.5 (191.0, 378.8)	101.0 (36.0, 351.5)	-3.332	0.001
B lymphocyte count (/ μ L)	109.3 (63.7, 183.3)	25.7 (9.4, 92.3)	-5.076	<0.001
Treg count (/ μ L)	0.259 (0.095, 0.516)	0.233 (0.135, 0.488)	-5.518	<0.001
Treg / CD4 ⁺ T lymphocyte Ratio	0.039 (0.028, 0.054)	0.021 (0.010, 0.038)	-4.418	<0.001
CD3 ⁺ CD4 ⁺ T lymphocyte count (/ μ L)	421.7 (258.6, 627.4)	38.9 (17.6, 168.3)	-6.256	<0.001
CD3 ⁺ CD4 ⁺ / T lymphocyte Ratio	0.292 (0.227, 0.340)	0.100 (0.054, 0.160)	-7.066	<0.001
CD3 ⁺ CD8 ⁺ T lymphocyte count (/ μ L)	241.1 (139.5, 488.6)	26.3 (7.2, 135.9)	-5.287	<0.001
CD3 ⁺ CD8 ⁺ / T lymphocyte Ratio	0.157 (0.126, 0.229)	0.053 (0.026, 0.104)	-5.719	<0.001

All the data in Supplemental table 3 are represented as the median [IQR]; IQR: Interquartile Range; CD: cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; Treg, regulatory T.

Supplemental Table 4. The flow cytometry results of cell counts and ratios of the CA patients on admission based on 28-day survival

	Survivors (n=20)	Non-survivors (n=65)	Z-value	P-value
T lymphocyte count (/μL)	502.0 (353.8, 1199.8)	514.0 (282.5, 1891.0)	-0.186	0.852
NK cell count (/μL)	167.0 (29.8, 309.3)	100.0 (36.0, 404.0)	-0.218	0.828
B lymphocyte count (/μL)	38.6 (15.7, 103.5)	19.2 (7.1, 65.7)	-0.632	0.527
Tregs count (/μL)	0.318 (0.145, 0.552)	0.212 (0.128, 0.479)	-0.611	0.396
Treg / CD4 ⁺ T lymphocyte Ratio	0.025 (0.009, 0.043)	0.021 (0.010, 0.034)	-0.498	0.619
CD3 ⁺ CD4 ⁺ T lymphocyte count (/μL)	55.1 (32.4, 228.0)	38.0 (16.0, 168.1)	-0.850	0.396
CD3 ⁺ CD4 ⁺ / T lymphocyte Ratio	0.118 (0.070, 0.236)	0.097 (0.049, 0.142)	-1.565	0.118
CD3 ⁺ CD8 ⁺ T lymphocyte count (/μL)	25.4 (12.5, 96.2)	26.3 (6.3, 138.8)	-0.021	0.983
CD3 ⁺ CD8 ⁺ / T lymphocyte Ratio	0.054 (0.033, 0.104)	0.053 (0.025, 0.104)	-0.187	0.852

All the data in Supplemental table 4 are represented as the median [IQR]; IQR: Interquartile Range; CD: cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; Treg, regulatory T.

Supplemental Table 5. The flow cytometry results of GR expression in the CA group and successful resuscitation group.

	Healthy Control Group (n=40)	Successful Resuscitation Group (n=85)	Z-value	P-value
Percentage of GR on B lymphocytes	0.963 (0.885, 0.992)	0.896 (0.605, 0.949)	-3.742	<0.001
MFI of GR on B lymphocytes	2.48 (1.91, 3.31)	1.73 (1.50, 2.37)	-3.980	<0.001
Percentage of GR on T lymphocytes	0.964 (0.889, 0.986)	0.900 (0.703, 0.955)	-3.755	<0.001
MFI of GR on T lymphocytes	2.98(1.95, 3.68)	1.92 (1.36, 1.99)	-3.853	<0.001
Percentage of GR on NK cells	0.907 (0.624, 0.983)	0.611 (0.306, 0.840)	-3.792	<0.001
MFI of GR on NK cells	2.19 (1.48, 2.96)	1.60 (1.36, 1.99)	-3.171	0.002
Percentage of GR on Treg cells	0.848 (0.680, 0.978)	0.784 (0.589, 0.911)	-1.837	0.066
MFI of GR on Treg cells	2.12 (1.53, 2.88)	1.76 (1.44, 2.30)	-1.990	0.047
Percentage of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	0.980 (0.874, 0.996)	0.957 (0.824, 0.985)	-2.204	0.100
MFI of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	2.65 (1.75, 3.38)	2.17 (1.70, 2.92)	-1.646	0.027
Percentage of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	0.986 (0.868, 0.996)	0.938 (0.823, 0.979)	-2.758	0.006
MFI of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	2.73 (1.73, 3.02)	2.10 (1.68, 2.54)	-2.668	0.008

All the data in Supplemental table 5 are represented as the median [IQR]. Abbreviations: IQR, interquartile range; CD, cluster-of-differentiation; NK, natural killer; Treg, regulatory T; GR, Glucocorticoid receptor; MFI, mean fluorescence intensity.

Supplemental Table 6. The flow cytometry results of GR expression in the survivors and non-survivors.

	Survivors (n=20)	Non-survivors (n=65)	Z-value	P-value
Percentage of GR on B lymphocytes	0.904 (0.595, 0.976)	0.906 (0.657, 0.946)	-0.787	0.431
MFI of GR on B lymphocytes	1.92 (1.52, 2.54)	1.72 (1.51, 2.31)	-0.881	0.378
Percentage of GR on T lymphocytes	0.899 (0.778, 0.969)	0.913 (0.692, 0.951)	-1.057	0.291
MFI of GR on T lymphocytes	2.05 (1.67, 2.83)	1.91 (1.64, 2.46)	-1.031	0.303
Percentage of GR on NK cells	0.717 (0.292, 0.886)	0.556 (0.302, 0.823)	-0.756	0.449
MFI of GR on NK cells	1.54 (1.37, 2.09)	1.61 (1.34, 1.87)	-0.565	0.572
Percentage of GR on Tregs	0.780 (0.667, 0.849)	0.799 (0.576, 0.923)	-0.440	0.660
MFI of GR on Tregs	1.61 (1.48, 2.30)	1.77 (1.45, 2.27)	-0.005	0.996
Percentage of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	0.975 (0.876, 0.985)	0.957 (0.845, 0.987)	-0.617	0.538
MFI of GR on CD3 ⁺ CD4 ⁺ T lymphocytes	2.08 (1.72, 3.35)	2.22 (1.71, 2.69)	-0.865	0.387
Percentage of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	0.963 (0.816, 0.977)	0.938 (0.834, 0.980)	-0.254	0.800
MFI of GR on CD3 ⁺ CD8 ⁺ T lymphocytes	2.08 (1.68, 3.10)	2.11(1.71, 2.46)	-0.653	0.514

All the data in Supplemental table 6 are represented as the median [IQR]. Abbreviations: IQR, Interquartile Range; CD, Cluster-of-differentiation; NK, natural killer; Treg, regulatory T; GR, glucocorticoid receptor; MFI, mean fluorescence intensity.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	Supplemental Figure 1
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-9, Supplemental Figure 1
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5,6,8,9
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5, 6, 8, Supplemental Figure 1
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 8
Bias	9	Describe any efforts to address potential sources of bias	6-8
Study size	10	Explain how the study size was arrived at	Supplemental Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8, 11

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(b) Describe any methods used to examine subgroups and interactions	N/A
(c) Explain how missing data were addressed	8, 11
(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	11
(e) Describe any sensitivity analyses	

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60**Results**

Participants	13 *	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Supplementa l Figure 1
		(b) Give reasons for non-participation at each stage	9,12, Supplementa l Figure 1
		(c) Consider use of a flow diagram	Supplementa l Figure 1
Descriptive data	14 *	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	Supplementa l Figure 1
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15 *	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	9-12
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-12, Electronic supplemental material
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A

Discussion

Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-14
Generalisability	21	Discuss the generalisability (external validity) of the study results	15

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17
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*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely

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2 available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at
3 <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is
4 available at www.strobe-statement.org.
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