

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [info.bmjopen@bmj.com](mailto:info.bmjopen@bmj.com)

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-060246
Article Type:	Original research
Date Submitted by the Author:	04-Jan-2022
Complete List of Authors:	Yu, Yanan; Capital Medical University, Department of Emergency Medicine Tang, Ziren; Capital Medical University, Department of Emergency Medicine Xie, Miaorong; Capital Medical University, Department of Emergency Medicine Li, Jiabao; Capital Medical University, Department of Critical Care Hang, Chen-Chen; Beijing Chao-Yang Hospital, Emergency Medicine An, Le; Capital Medical University, Department of Emergency Medicine Li, Chunsheng; Beijing Chaoyang Hospital, Department of Emergency Medicine
Keywords:	ACCIDENT & EMERGENCY MEDICINE, INTENSIVE & CRITICAL CARE, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

SCHOLARONE™  
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

---

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

3 Yanan Yu<sup>1</sup>; Ziren Tang<sup>1</sup>; Miaorong Xie<sup>2</sup>; Jiabao Li<sup>3</sup>; Chenchen Hang<sup>1</sup>; Le An<sup>1</sup>;  
4 Chunsheng Li<sup>1</sup>, \*

5 <sup>1</sup>Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical  
6 University, Beijing 100020, China

7 <sup>2</sup>Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical  
8 University, Beijing 100032, China

9 <sup>3</sup>Department of Critical Care, Beijing Friendship Hospital, Capital Medical University,  
10 Beijing 100020, China

11 \*Corresponding author: Chungsheng Li, M.D.

12 Department of Emergency Medicine, Beijing Chaoyang Hospital,  
13 Capital Medical University, 8 Worker's Stadium South Road, Chaoyang District,  
14 Beijing 100020, China, Tel: [+86 13681392380](tel:+8613681392380); E-mail: [lcscy@163.com](mailto:lcscy@163.com)

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-

1  
2  
3  
4 45 survivors.

5  
6 46 **Conclusions:** This study revealed that GR expression and cell counts rapidly decreased,  
7  
8  
9 47 whereas plasma total cortisol levels increased, in the early period after ROSC among  
10  
11 48 patients who experienced CA. Our findings provide insights into GC sensitivity and  
12  
13 49 immunosuppressive status in these patients, and a new perspective for GC targeted  
14  
15 50 treatment.  
16  
17

18  
19  
20 51 **Strengths and limitations of this study**

- 21  
22 52 1. Explore whether controversy over glucocorticoid use is associated with different  
23  
24 53 levels of glucocorticoid receptor expression in cardiac arrest patients for the first  
25  
26 54 time.  
27  
28  
29 55 2. Glucocorticoid receptor expression rapidly decreased in the early period following  
30  
31 56 restoration of spontaneous circulation among patients who experienced cardiac arrest .  
32  
33  
34 57 3. We only observed changes in glucocorticoid receptor expression of cardiac  
35  
36 58 arrest patients at the early period following restoration of spontaneous circulation, and  
37  
38 59 long-term dynamic observation would be helpful to understand the significance of  
39  
40 60 clinical steroid therapy.  
41  
42  
43  
44

45 61 **Introduction**

46  
47  
48 62 Cardiac arrest (CA) is an important health problem globally; about 356,500 people  
49  
50 63 experience medical emergencies due to CA in the United States, and over 544,000  
51  
52 64 people die from sudden CA in China annually. [1, 2] The systemic ischemia-reperfusion  
53  
54 65 response in patients who have experienced CA can present as post-CA syndrome  
55  
56 66 (PCAS) or systematic inflammatory response syndrome (SIRS), which increases the  
57  
58  
59  
60

1  
2  
3  
4 67 risk of multiple organ failure and infection and affects the inflammatory response and  
5  
6 68 prognosis of patients after the return of spontaneous circulation (ROSC). [3-6]  
7  
8

9 69 CA is the most intense among acute stress events, which seriously affect the function  
10  
11 70 of the pituitary and adrenal axis. [7] Studies have shown that abnormal cortisol levels  
12  
13 71 and relative adrenocortical insufficiency after ROSC in patients who experienced CA  
14  
15 72 are related to their prognosis. [8-11] However, the clinical application of  
16  
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  
20  
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  
28  
29 79 mechanisms. [12-17] Therefore, the role of GCs in the occurrence and development of  
30  
31 80 PCAS needs to be studied further.  
32  
33  
34  
35  
36  
37  
38  
39

40 81 GCs combine with intracellular GC receptors (GRs) to exert anti-inflammatory and  
41  
42 82 immunosuppressive effects and reduce the production as well as release of  
43  
44 83 inflammatory cytokines. [18, 19] The affinity of GRs to GCs in circulating monocytes  
45  
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  
49  
50 86 shock, and high serum cortisol level. [22] However, hitherto, no study has reported the  
51  
52 87 GR expression after ROSC in patients who experienced CA. Previous studies have  
53  
54 88 found that the counts of circulating B and T lymphocytes, regulatory T (Treg) cells, and  
55  
56  
57  
58  
59  
60

---

89 monocytes and expression of human leukocyte antigen DR (HLA-DR) on circulatory  
90 monocytes and B and T lymphocytes are reduced. [23, 24] Hence, the aim of this study  
91 was to investigate the relationship between GR expression and immune alteration in the  
92 early period after ROSC in patients who experienced CA by observing GR expression  
93 in circulatory T and B lymphocytes, NK cells, and Treg cells, their cell counts, and  
94 plasma total cortisol and adrenocorticotrophic hormone (ACTH) levels.

## 96 **MATERIALS AND METHODS**

### 97 **Study participants**

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

### 110 **Data collection**



1  
2  
3  
4 111 We collected data on demographics, resuscitation (initial heart rhythm, ROSC time,  
5  
6 112 and cumulative adrenaline epinephrine dose), and laboratory findings (routine blood  
7  
8  
9 113 cell counts, blood gas analysis, and blood biochemical tests performed 6 h after ROSC).  
10  
11 114 Acute Physiology and Chronic Health Evaluation (APACHE) II and the Sequential  
12  
13  
14 115 Organ Failure Assessment (SOFA) were used to determine disease severity. Residual  
15  
16  
17 116 samples of blood, with heparin anticoagulant, from routine clinical tests or physical  
18  
19  
20 117 health examinations were collected, maintained at 4 °C during transport and storage,  
21  
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  
26  
27  
28 120 and used to determine total cortisol and ACTH levels. During follow-up, 28-day  
29  
30 121 survival data were also collected. Supplemental Figure 1 shows the workflow of this  
31  
32  
33 122 study.

34  
35  
36  
37  
38 123

### 124 **Flow cytometry**

39  
40 125 GR expression in T and B lymphocytes, NK cells, and Treg cells was measured. Briefly,  
41  
42  
43 126 a 100- $\mu$ L peripheral blood sample was stained for 20 min with surface antibodies (CD3,  
44  
45  
46 127 CD4, CD8, CD19, CD16, CD56, CD25, and CD127) in a dark place. Erythrocytes were  
47  
48  
49 128 lysed for 15 min, and the debris was washed away. Before intracellular GR staining,  
50  
51  
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  
57  
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.

1  
2  
3  
4 133 According to the manufacturer's recommendations, all antibodies and their isotype  
5  
6 134 controls were used at a concentration of 1  $\mu$ L per 100  $\mu$ L of whole blood. Samples were  
7  
8  
9 135 measured using the Gallios flow cytometer (Beckman Coulter, Brea, CA, USA) and  
10  
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  
18  
19 139 gated by CD3<sup>+</sup>CD4<sup>+</sup> or CD3<sup>+</sup>CD8<sup>+</sup>, B cells were gated by CD3<sup>-</sup>CD19<sup>+</sup>, NK cells were  
20  
21  
22 140 gated by CD16<sup>+</sup>CD56<sup>+</sup>, and Tregs were gated by CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>. At least  
23  
24  
25 141 10,000 events were collected in the lymphocyte cell gate for each sample. Results are  
26  
27  
28 142 expressed as percentages and mean fluorescence intensity (MFI) values.

29  
30 143 Absolute CD3<sup>+</sup> and CD4<sup>+</sup> lymphocyte, NK cell, and Treg cell counts were obtained  
31  
32 144 using Flow-Count fluorospheres (Beckman Coulter, Catalogue No. 7547053),  
33  
34  
35 145 according to the manufacturer's instructions. B, CD3<sup>+</sup>CD4<sup>+</sup>T, CD3<sup>+</sup>CD8<sup>+</sup>T, and Treg  
36  
37  
38 146 cell counts were calculated by their percentages in CD3<sup>+</sup> or CD4<sup>+</sup> lymphocytes  
39  
40  
41 147 multiplied by CD3<sup>+</sup> or CD4<sup>+</sup> lymphocyte counts.

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

46  
47  
48 150 Venous blood samples were collected in ethylenediaminetetraacetic acid tubes,  
49  
50  
51 151 centrifuged 10 min at 3000 rpm, and then stored at -80 °C. Plasma total cortisol  
52  
53  
54 152 (IMMULITE 2000 Cortisol, L2KCO2, UK) and ACTH (IMMULITE 2000 ACTH,  
55  
56  
57 153 L2KAC2, UK) levels were assayed using a chemiluminescent immunoassay on a  
58  
59  
60 154 Siemens automated analyzer (IMMULITE 2000 XPi; Siemens Healthcare Diagnostics,

---

1  
2  
3  
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  
7  
8  
9 157 5.00 pg/mL.  
10  
11  
12 158

### 13 14 159 **Statistical analyses**

15  
16  
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  
20  
21 162 deviations. Since the data for total cortisol and ACTH levels had a skewed distribution,  
22  
23 163 we compared our results with the natural logarithmic conversion values after adding 1  
24  
25 164 ( $\ln [\text{total cortisol} + 1]$ ,  $\ln [\text{ACTH} + 1]$ ). Measurement data with a skewed distribution are  
26  
27 165 expressed as medians (25th and 75th percentiles). The Mann–Whitney U test was used  
28  
29 166 to compare variables between groups. The qualitative parameters in the  $2 \times 2$   
30  
31 167 contingency table were used for analysis. All statistical tests were two-tailed, and a P-  
32  
33 168 value of  $<0.05$  was considered statistically significant.  
34  
35  
36  
37  
38  
39  
40  
41  
42

### 43 170 **Follow-up**

44  
45 171 Patients who experienced CA were classified into survivor and non-survivor groups  
46  
47 172 according to the 28-day survival endpoint. Those with all-cause mortality within the  
48  
49 173 follow-up period were considered non-survivors. If data were lost, the corresponding  
50  
51 174 candidate was excluded.  
52  
53  
54  
55  
56  
57  
58  
59  
60

### 176 **Patient and public involvement**

1  
2  
3  
4 177 This study was approved by the Medical Ethics Committee (2013-KE-1). Patient  
5  
6 178 consent to participate was obtained prior to enrolment in this study.  
7  
8  
9 179

## 10 11 180 **Results**

### 12 13 181 **Patient characteristics**

14  
15  
16  
17 182 In total, 40 healthy individuals and 85 patients who experienced CA were analyzed.  
18  
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  
28  
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  
32  
33 190 ( $P < 0.001$  for all) and survivor and non-survivor groups ( $P < 0.001$  and  $P = 0.011$ ,  
34  
35 191 respectively).  
36  
37  
38  
39  
40  
41  
42  
43  
44

45 193 **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.

1  
2  
3  
4 199

5  
6 **200 Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts after**  
7  
8  
9 **201 ROSC**

10  
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).  
14  
15 204 Additionally, the  $CD3^+CD4^+/T$  lymphocyte,  $CD3^+CD8^+/T$  lymphocyte, and Treg  
16  
17 205 cell/ $CD4^+$  T lymphocyte ratios were significantly lower after ROSC in patients who  
18  
19 206 experienced CA than in healthy controls ( $P < 0.001$  for all) (Fig. 1; Supplemental Table  
20  
21  
22 207 3). However, there were no significant differences in these cell counts and ratios  
23  
24  
25 208 between survivors ( $n=20$ ) and non-survivors ( $n=65$ ) ( $P > 0.05$  for all) (Supplemental  
26  
27  
28 209 Table 4).  
29  
30  
31  
32  
33  
34

35 **211 GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells after**  
36  
37 **212 ROSC**

38  
39  
40 213 The MFI and percentages of GR expression in B and T lymphocytes, NK cells, and  
41  
42 214  $CD3^+CD8^+$  T lymphocytes were significantly lower after ROSC in patients who  
43  
44 215 experienced CA than in healthy individuals ( $P < 0.01$  for all) (Fig. 2A–D, G, H, K, L).  
45  
46  
47 216 There were also significant reductions in the MFI in Treg cells and  $CD3^+CD4^+$  T  
48  
49 217 lymphocytes ( $P < 0.05$  for all) (Figs. 2E, I) but not in the percentages of GR expression  
50  
51 218 ( $P > 0.05$  for all) (Figs. 2F, J; Supplemental Table 5). However, there were no significant  
52  
53  
54 219 differences in the MFI and percentages of GR expression in these cells between  
55  
56  
57 220 survivors and non-survivors ( $P > 0.05$  for all) (Supplemental Table 6).  
58  
59  
60

221

## 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/ $\text{CD4}^+$  T lymphocyte ratios were significantly reduced after ROSC. NK cells,  
242 which are special innate immune cells that have cytotoxic functions similar to

1  
2  
3  
4 243 CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes, mainly distinguish infected and stressed cells from healthy  
5  
6 244 cells and eliminate intracellular infection as well as dysfunctional cells. [27, 28] T  
7  
8  
9 245 lymphocytes are also important because of their function as adaptive immune cells for  
10  
11 246 the control and elimination of infection. [27] Moreover, B and T lymphocytes mediate  
12  
13  
14 247 humoral and cellular immunity, respectively. This study was performed at an earlier  
15  
16  
17 248 period and involved a more comprehensive assessment of the immune system of  
18  
19 249 patients who experienced CA, and our findings more substantially supported the rapid  
20  
21  
22 250 emergence of immune dysfunction in these patients after ROSC than previous reports.  
23  
24  
25

26 251 The effectiveness of GC use in these patients during and after resuscitation has been  
27  
28 252 controversial due to insufficient evidence. However, the use of GCs during resuscitation  
29  
30  
31 253 improves the survival rate of patients who experience CA due to its direct anti-  
32  
33  
34 254 inflammatory, immunosuppressive effects, hemodynamics, and positive inotropic  
35  
36 255 effects. All of this ultimately leads to an increased stress capacity of the body. [18, 19]  
37  
38  
39 256 GCs can activate GRs in cells when the body is under stress, thereby increasing both  
40  
41 257 the effectiveness of resuscitation and discharge survival rate. This study is the first to  
42  
43  
44 258 explore GR expression in circulating immune cells in patients who experienced CA  
45  
46  
47 259 after ROSC. We observed that GR expression in B and T lymphocytes, NK cells, and  
48  
49 260 CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes decreased significantly in patients who experienced CA,  
50  
51  
52 261 whereas the percentage of GR<sup>+</sup> Treg cells and CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes showed a  
53  
54 262 slight decrease. Moreover, we observed a more significant decrease in the MFI of GR  
55  
56  
57 263 expression in Treg cells and CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes but not in the percentage of GR  
58  
59  
60



---

1  
2  
3  
4 264 expression. Previous studies have found decreased expression of GRs in peripheral  
5  
6 265 polymorphonuclear cells in critically ill patients, [21] and antagonism to GRs  
7  
8  
9 266 aggravates viral and bacterial infections. [29] The results of this study suggest that the  
10  
11 267 decrease in intracellular GR expression in patients who experienced CA is one of the  
12  
13  
14 268 causes of GC resistance, due to insufficient binding of GRs and GCs, GC insensitivity,  
15  
16  
17 269 and the inability of GCs to effectively exert anti-inflammatory and immunosuppressive  
18  
19  
20 270 effects. These findings may also explain why different results regarding the clinical  
21  
22 271 application of GCs have been reported previously and support the possibility of using  
23  
24  
25 272 GCs in the clinical treatment of patients who experienced CA.

26  
27 273 We also found that the total plasma cortisol levels were significantly higher in  
28  
29  
30 274 patients who experienced CA, but ACTH levels were not. High levels of inflammatory  
31  
32  
33 275 cytokines inhibit ACTH release. [18] During critical illness, the body does not  
34  
35  
36 276 sufficiently metabolize cortisol. [30] In addition, the continuous increase in plasma  
37  
38  
39 277 cortisol levels may trigger the negative feedback pathway of the hypothalamic-  
40  
41  
42 278 pituitary-adrenal axis, inhibiting the release of ACTH and cortisol and eventually  
43  
44  
45 279 leading to adrenal insufficiency. These factors may explain the opposite trends of  
46  
47  
48 280 plasma ACTH and cortisol levels in the patients who were included in this study and  
49  
50  
51 281 experienced CA. Notably, this result suggests that low GR expression levels are not  
52  
53  
54 282 matched with high plasma total cortisol levels. Previous studies have found that GC use  
55  
56  
57 283 during resuscitation may benefit patients who experience CA. [13-16] The benefits,  
58  
59  
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

1  
2  
3  
4 286 a high dose of GCs, or GCs may work through other non-GR pathways. It is also  
5  
6 287 possible that the immune function of patients who experience CA is suppressed due to  
7  
8  
9 288 ischemia-reperfusion injury, which requires a large dose of GCs to stimulate GRs to  
10  
11  
12 289 function. This study did not provide data on plasma GC levels and GR expression in a  
13  
14 290 group of patients who were administered GCs and successfully resuscitated; therefore,  
15  
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

1  
2  
3  
4 308 about GC sensitivity and immunosuppressive status in these patients. In addition, this  
5  
6 309 study provides a new perspective for clinical targeted treatment using GCs and high-  
7  
8  
9 310 quality prognosis in CA patients.  
10

11 311

12  
13  
14 312 **Acknowledgements:** We thank all researchers who participated in this study and all  
15  
16  
17 313 colleagues in the emergency department who provided support.  
18

19 314 **Ethics Approval:** This study was approved by the Medical Ethics Committee of  
20  
21  
22 315 Beijing Chaoyang Hospital (2013-KE-1). Patient consent to participate was obtained  
23  
24  
25 316 prior to enrolment in this study.  
26

27 317 **Contributorship statement:** CL designed the study and reviewed the manuscript.  
28  
29  
30 318 YNY searched the literature and contributed to the experimental studies, data  
31  
32  
33 319 analysis, and writing of the manuscript. ZRT, CCH, and LA collected and analyzed  
34  
35 320 data. JBL and MRX helped with the statistical analyses. All authors have read and  
36  
37  
38 321 approved the final manuscript.  
39

40 322 **Competing interests:** All authors declare no competing interest associated with this  
41  
42  
43 323 project.  
44

45 324 **Funding:** This research received no specific grant from any funding agency in the  
46  
47  
48 325 public, commercial or not-for-profit sectors.  
49

50 326 **Data sharing statement:** All data relevant to the study are included in the article or  
51  
52  
53 327 uploaded as supplementary information. Due to privacy and ethical concerns, data can  
54  
55  
56 328 not be shared.  
57

58 329  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

---

330

331

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.

- 
- 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, Stefaniotou A, Varvaroussis 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

- 
- 1  
2  
3  
4 398 return of spontaneous circulation. *Scand J Immunol* 2018;**88**:e12707.
- 5  
6 399 25 Perkins GD, Travers AH, Berg RA, et al. Resuscitation-Part 3: adult basic life  
7  
8 support and automated external defibrillation: 2015 International Consensus on  
9  
10 400  
11  
12 401 Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science with  
13  
14 402 Treatment Recommendations. *Resuscitation* 2015;**95**:e43–e69.
- 15  
16 403 26 Beurskens CJ, Horn J, de Boer AM, et al. Cardiac arrest patients have an impaired  
17  
18 immune response, which is not influenced by induced hypothermia. *Crit Care*  
19  
20 404  
21  
22 405 2014;**18**:R162.
- 23  
24 406 27 Lanier LL. NK cell recognition. *Annu Rev Immunol* 2005;**23**:225–74.
- 25  
26 407 28 Vivier E, Tomasello E, Baratin M et al. Functions of natural killer cells. *Nat*  
27  
28  
29 408  
30  
31  
32 409 29 Webster JI, Sternberg EM. Role of the hypothalamic-pituitary-adrenal axis,  
33  
34 glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial  
35  
36 410  
37  
38 411 and viral products. *J Endocrinol* 2004;**181**:207–21.
- 39  
40 412 30 Boonen E, Vervenne H, Meersseman P, et al. Reduced cortisol metabolism during  
41  
42  
43 413  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



---

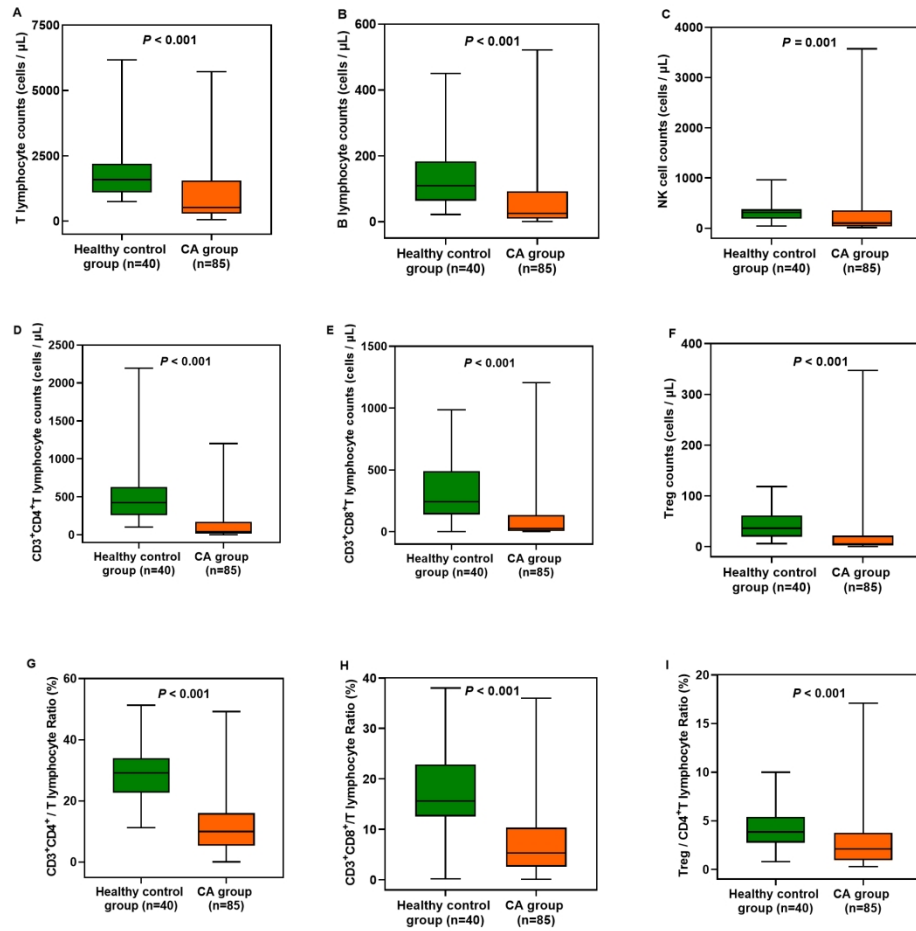
1  
2  
3  
4 414 **Figure legends**

5  
6 415 **Fig. 1.** Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and  
7  
8  
9 416 CD3<sup>+</sup>CD4<sup>+</sup>/T, CD3<sup>+</sup>CD8<sup>+</sup>/T, and Treg/CD4<sup>+</sup>T lymphocyte ratios between the healthy  
10  
11 417 control group and CA group. The CA group showed significant differences compared  
12  
13  
14 418 with the healthy control group (P<0.001). CA, cardiac arrest; CD, cluster-of-  
15  
16  
17 419 differentiation; NK, natural killer; Treg, regulatory T.

18  
19 420 **Fig. 2.** Expression of GRs in circulatory T and B lymphocytes, NK cells, and Treg cells  
20  
21  
22 421 in the healthy control group and CA group. The CA group showed significant  
23  
24  
25 422 differences compared with the healthy control group (P<0.05). CA, cardiac arrest; CD,  
26  
27 423 cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC,  
28  
29  
30 424 return of spontaneous circulation; Treg, regulatory T.

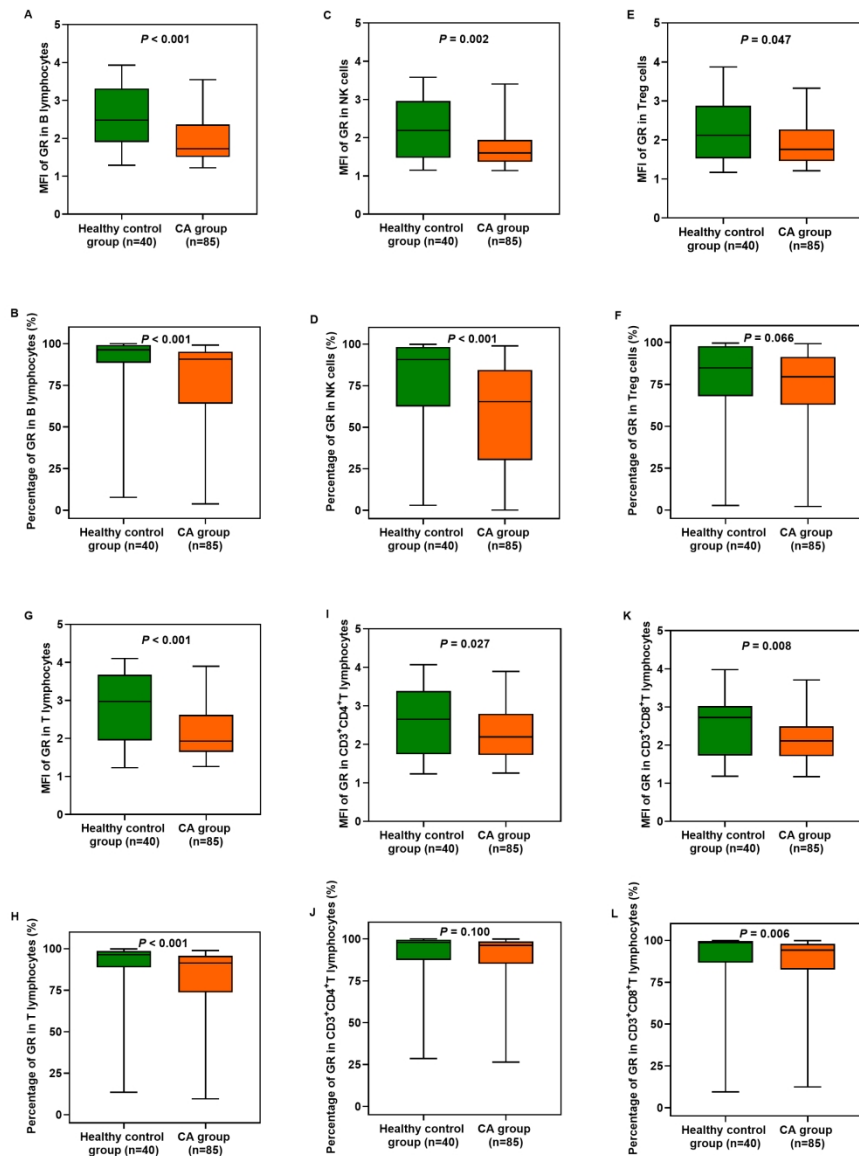
31  
32 425 **Fig. 3.** (A, B) Plasma total cortisol and ACTH levels (the natural logarithmic  
33  
34  
35 426 conversion values after adding 1) after ROSC in the healthy control group and CA  
36  
37  
38 427 group. (C, D) Plasma total cortisol and ACTH levels in survivors and non-survivors  
39  
40  
41 428 after ROSC. The CA group showed significant differences compared with the healthy  
42  
43  
44 429 control group (P<0.05). ACTH, adrenocorticotrophic hormone; CA, cardiac arrest;  
45  
46  
47 430 ROSC, return of spontaneous circulation.

48 431  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



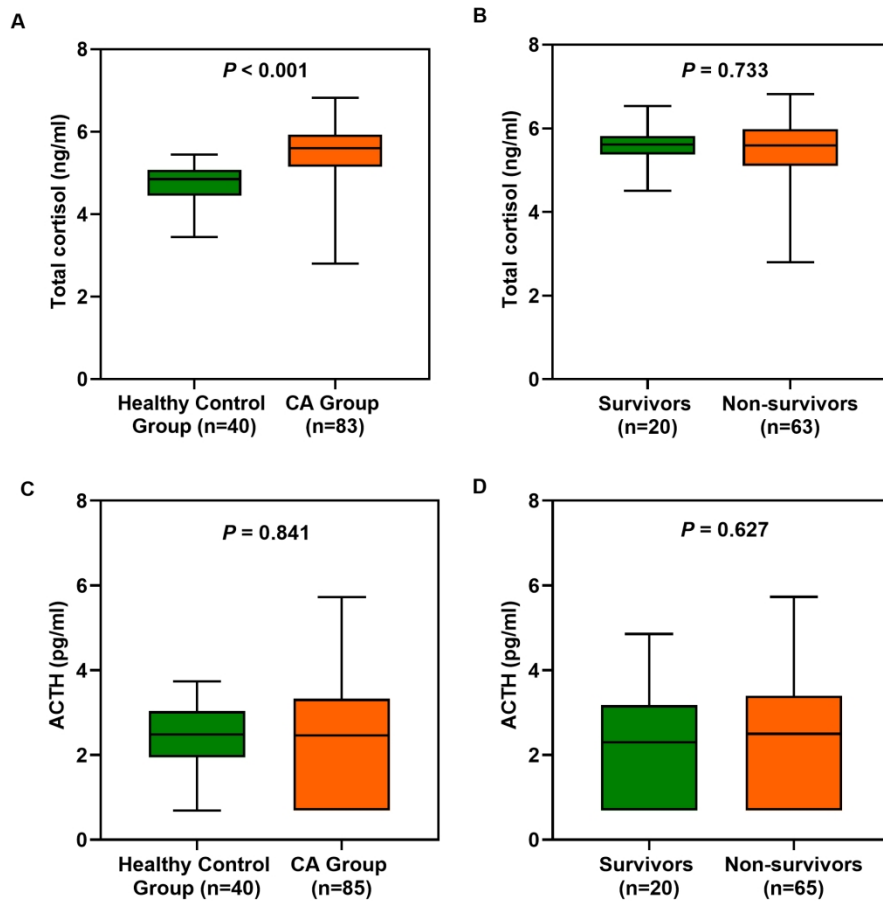
Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and CD3+CD4+/T, CD3+CD8+/T, and Treg/CD4+T lymphocyte ratios between the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group (P<0.001). CA, cardiac arrest; CD,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

Yanan Yu<sup>1</sup>; Ziren Tang<sup>1</sup>; Miaorong Xie<sup>2</sup>; Jiabao Li<sup>3</sup>; Chenchen Hang<sup>1</sup>; Le An<sup>1</sup>; Chunsheng Li<sup>1, \*</sup>

<sup>1</sup>Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

<sup>2</sup>Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing 100032, China

<sup>3</sup>Department of Critical Care, Beijing Friendship Hospital, Capital Medical University, Beijing 100020, China

\*Corresponding author: Chungsheng Li, M.D.

Department of Emergency Medicine, Beijing Chaoyang Hospital,

Capital Medical University, 8 Worker's Stadium South Road, Chaoyang District, Beijing 100020, China, Tel: [+86](tel:+8613681392380)

[13681392380](tel:+8613681392380); E-mail: [lcscy@163.com](mailto:lcscy@163.com)

#### Contents

Supplemental Figure 1

Supplemental Table 1

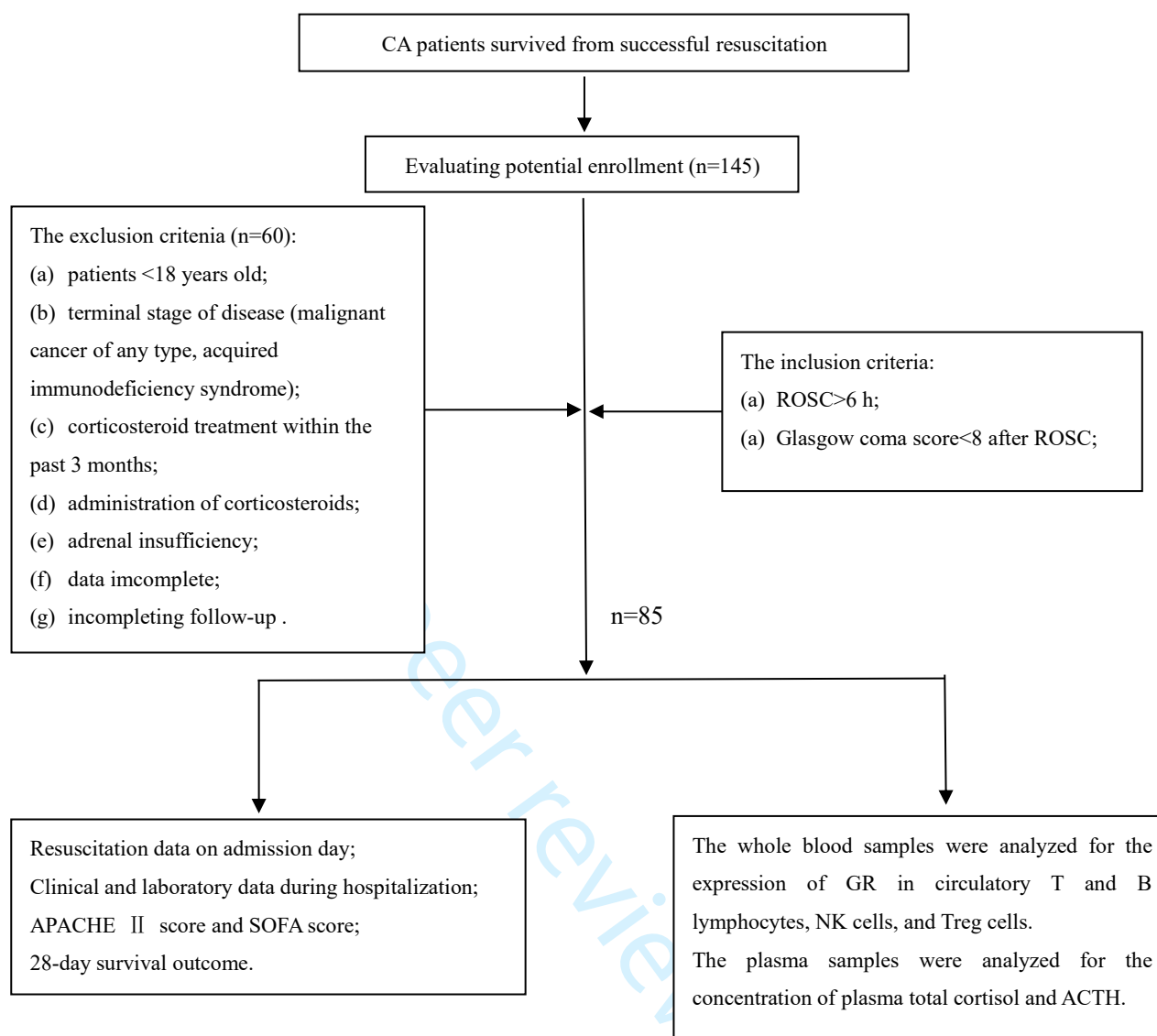
Supplemental Table 2

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 <sup>a</sup>
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 <sup>b</sup>
Mouse IgG1 Isotype	MCA928F	FITC	Bio-Rad
Mouse IgG1, $\kappa$ Isotype	557872	PE-Cy7	BD Pharmingen
Mouse IgG1, $\kappa$ Isotype	554680	PE	BD Pharmingen
Mouse IgG1, $\kappa$ Isotype	558120	Pacific Blue	BD Pharmingen

<sup>a</sup> BD Pharmingen, San Diego, USA; <sup>b</sup> 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 / $\mu$ L)	1586.0 (1101.5, 2192.5)	514.0 (287.5, 1555.0)	-4.515	<0.001
NK cell count (/ $\mu$ L)	311.5 (191.0, 378.8)	101.0 (36.0, 351.5)	-3.332	0.001
B lymphocyte count (/ $\mu$ L)	109.3 (63.7, 183.3)	25.7 (9.4, 92.3)	-5.076	<0.001
Treg count (/ $\mu$ L)	0.259 (0.095, 0.516)	0.233 (0.135, 0.488)	-5.518	<0.001
Treg / CD4 <sup>+</sup> T lymphocyte Ratio	0.039 (0.028, 0.054)	0.021 (0.010, 0.038)	-4.418	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocyte count (/ $\mu$ L)	421.7 (258.6, 627.4)	38.9 (17.6, 168.3)	-6.256	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup> / T lymphocyte Ratio	0.292 (0.227, 0.340)	0.100 (0.054, 0.160)	-7.066	<0.001
CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocyte count (/ $\mu$ L)	241.1 (139.5, 488.6)	26.3 (7.2, 135.9)	-5.287	<0.001
CD3 <sup>+</sup> CD8 <sup>+</sup> / 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 <sup>+</sup> T lymphocyte Ratio	0.025 (0.009, 0.043)	0.021 (0.010, 0.034)	-0.498	0.619
CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocyte count (/μL)	55.1 (32.4, 228.0)	38.0 (16.0, 168.1)	-0.850	0.396
CD3 <sup>+</sup> CD4 <sup>+</sup> / T lymphocyte Ratio	0.118 (0.070, 0.236)	0.097 (0.049, 0.142)	-1.565	0.118
CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocyte count (/μL)	25.4 (12.5, 96.2)	26.3 (6.3, 138.8)	-0.021	0.983
CD3 <sup>+</sup> CD8 <sup>+</sup> / 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.

	<b>Healthy Control Group (n=40)</b>	<b>Successful Resuscitation Group (n=85)</b>	<b>Z-value</b>	<b>P-value</b>
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 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	0.980 (0.874, 0.996)	0.957 (0.824, 0.985)	-2.204	0.100
MFI of GR on CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	2.65 (1.75, 3.38)	2.17 (1.70, 2.92)	-1.646	0.027
Percentage of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocytes	0.986 (0.868, 0.996)	0.938 (0.823, 0.979)	-2.758	0.006
MFI of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> 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 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	0.975 (0.876, 0.985)	0.957 (0.845, 0.987)	-0.617	0.538
MFI of GR on CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	2.08 (1.72, 3.35)	2.22 (1.71, 2.69)	-0.865	0.387
Percentage of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocytes	0.963 (0.816, 0.977)	0.938 (0.834, 0.980)	-0.254	0.800
MFI of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> 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
<b>Introduction</b>			
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
<b>Methods</b>			
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	

Continued on next page

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

<b>Results</b>			
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	11, 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	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
<b>Discussion</b>			
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
<b>Other information</b>			
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

\*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](http://www.strobe-statement.org).  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-060246.R1
Article Type:	Original research
Date Submitted by the Author:	24-May-2022
Complete List of Authors:	Yu, Yanan; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine Tang, Ziren; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine Xie, Miaorong; Capital Medical University Affiliated Beijing Friendship Hospital, Department of Emergency Medicine Li, Jiabao; Capital Medical University Affiliated Beijing Friendship Hospital, Department of Critical Care Hang, Chen-Chen; Beijing Chao-Yang Hospital, Emergency Medicine An, Le; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine Li, Chunsheng; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine
<b>Primary Subject Heading</b>:	Emergency medicine
Secondary Subject Heading:	Intensive care
Keywords:	ACCIDENT & EMERGENCY MEDICINE, INTENSIVE & CRITICAL CARE, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

SCHOLARONE™  
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

---

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**

4 Yanan Yu<sup>1</sup>; Ziren Tang<sup>1</sup>; Miaorong Xie<sup>2</sup>; Jiabao Li<sup>3</sup>; Chenchen Hang<sup>1</sup>; Le An<sup>1</sup>;  
5 Chunsheng Li<sup>1</sup>,\*

6 <sup>1</sup>Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical  
7 University, Beijing 100020, China

8 <sup>2</sup>Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical  
9 University, Beijing 100032, China

10 <sup>3</sup>Department of Critical Care, Beijing Friendship Hospital, Capital Medical University,  
11 Beijing 100020, China

12 \*Corresponding author: Chungsheng Li, M.D.

13 Department of Emergency Medicine, Beijing Chaoyang Hospital,  
14 Capital Medical University, 8 Worker's Stadium South Road, Chaoyang District,  
15 Beijing 100020, China, Tel: [+86 13681392380](tel:+8613681392380); E-mail: [lcseyyy@163.com](mailto:lcseyyy@163.com)

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-

1  
2  
3  
4 45 survivors.

5  
6 46 **Conclusions:** This study revealed that GR expression and cell counts rapidly decreased,  
7  
8  
9 47 whereas plasma total cortisol levels increased in the early period after ROSC among  
10  
11  
12 48 patients who experienced CA. Our findings provide important information about GR  
13  
14  
15 49 level and function, and immunosuppressive status in these patients. Assessing GR  
16  
17  
18 50 expression in CA patients may help screening for those who are more sensitive to  
19  
20  
21 51 glucocorticoid therapy.

22  
23  
24  
25 53 **Strengths and limitations of this study**

- 26  
27 54 1. The study design will be single-center, prospective.  
28  
29  
30 55 2. This is the first study to evaluate the GR expression in the early period following  
31  
32  
33 56 ROSC among CA patients.  
34  
35  
36 57 3. Only CA patients in the early period following ROSC will be included, limiting the  
37  
38  
39 58 generalisability of the results.  
40  
41  
42 59 4. Decreased GR expression may affect the sensitivity of CA patients to GCs.  
43  
44  
45 60 5. Decreased GR expression may affect potential immune consequences of CA  
46  
47  
48 61 patients.

49  
50  
51 63 **Introduction**

52  
53 64 Cardiac arrest (CA) is a significant health problem globally; about 356,500 people  
54  
55  
56 65 experience medical emergencies due to CA in the United States, and over 544,000  
57  
58  
59 66 people die from sudden CA in China annually. [1, 2] The systemic ischemia-reperfusion  
60

1  
2  
3  
4 67 response in patients who have experienced CA can present as post-cardiac arrest  
5  
6 68 syndrome (PCAS) or systematic inflammatory response syndrome (SIRS), which  
7  
8  
9 69 increases the risk of multiple organ failure and infection and affects the inflammatory  
10  
11  
12 70 response and prognosis of patients after the return of spontaneous circulation (ROSC).  
13  
14 71 [3-6]

17 72 CA is the most intense among acute stress events, which seriously affect the pituitary  
18  
19 73 and adrenal axis function. [7] Studies have shown that abnormal cortisol levels and  
20  
21  
22 74 relative adrenocortical insufficiency after ROSC in patients who experienced CA are  
23  
24  
25 75 related to their prognosis. [8-11] However, the clinical application of glucocorticoids  
26  
27 76 (GCs) is controversial. In the 2015 International Cardiopulmonary Resuscitation  
28  
29  
30 77 Guidelines, the routine use of GCs is not recommended for the resuscitation of patients  
31  
32  
33 78 with in-hospital or out-of-hospital CA. [12] Recent clinical studies have shown that  
34  
35  
36 79 early administration of corticosteroids after CA can improve the success rate of ROSC,  
37  
38 80 nervous system functional outcome, and prognosis, which is speculated to be related to  
39  
40  
41 81 its influence on hemodynamics, and SIRS response, and other mechanisms. [12-17]  
42  
43 82 Therefore, the role of GCs in the occurrence and development of PCAS needs to be  
44  
45  
46 83 studied further.

48 84 GCs combine with intracellular GC receptors (GRs) to exert anti-inflammatory and  
49  
50  
51 85 immunosuppressive effects and reduce the production and the release of inflammatory  
52  
53  
54 86 cytokines. [18, 19] The affinity of GRs to GCs in circulating monocytes is decreased in  
55  
56  
57 87 patients with acquired immunodeficiency syndrome. [20] The expression of GR alpha  
58  
59 88 and beta in peripheral polymorphonuclear cells is decreased in patients with critical  
60

1  
2  
3  
4 89 illness, [21] pediatric septic shock, and high serum cortisol levels. [22] However, no  
5  
6 90 study has reported the GR expression after ROSC in patients who experienced CA.  
7  
8  
9 91 Previous studies have found that the counts of circulating B and T lymphocytes,  
10  
11 92 regulatory T (Treg) cells, and monocytes and expression of human leukocyte antigen  
12  
13 93 DR (HLA-DR) on circulatory monocytes and B and T lymphocytes are reduced. [23,  
14  
15 94 24] Hence, this study aimed to investigate the relationship between GR expression and  
16  
17 95 immune alteration in the early period after ROSC in patients who experienced CA by  
18  
19 96 observing GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells,  
20  
21 97 their cell counts, and total plasma cortisol and adrenocorticotrophic hormone (ACTH)  
22  
23 98 levels.  
24  
25  
26  
27  
28  
29  
30  
31  
32

## 33 **Materials and methods**

### 34 **Study participants**

35  
36  
37 102 This was an observational study conducted in the Emergency Department (ED).  
38  
39 103 According to the 2015 International Cardiopulmonary Resuscitation Guidelines, [25]  
40  
41 104 we enrolled patients in the early ROSC period after CA (both in-hospital and out-of-  
42  
43 105 hospital CA) and were admitted to the ED between October 2018 and October 2019.  
44  
45  
46 106 The inclusion criteria were patients with CA > 6 and < 24 hours after ROSC, with a  
47  
48 107 Glasgow coma score < 8. The exclusion criteria were (a) <18 years of age, (b) terminal  
49  
50 108 stage of disease (such as cancer of any type, acquired immunodeficiency syndrome),  
51  
52 109 (c) corticosteroid treatment within the past three months, (d) administration of  
53  
54 110 corticosteroids, and (e) adrenal insufficiency. All patients were treated according to the  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 111 2015 International Cardiopulmonary Resuscitation Consensus. [13] After a physical  
5  
6 112 examination, age- and sex-matched healthy individuals were recruited for the control  
7  
8  
9 113 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- $\mu$ L peripheral blood



1  
2  
3  
4 133 sample was stained for 20 min with surface antibodies (CD3, CD4, CD8, CD19, CD16,  
5  
6 134 CD56, CD25, and CD127) in a dark place. Erythrocytes were lysed for 15 min, and the  
7  
8  
9 135 debris was washed away. Before intracellular GR staining, surface-stained cells were  
10  
11  
12 136 fixed and permeabilized using the BD Transcription Factor Buffer Set (BD  
13  
14 137 Pharmingen, San Diego, USA, Catalogue No. 562574). Monoclonal antibodies and  
15  
16  
17 138 their isotype controls were all purchased from BD Biosciences (San Jose, CA, USA).  
18  
19 139 Details of all antibodies are shown in Supplemental Table 1. According to the  
20  
21  
22 140 manufacturer's recommendations, all antibodies and their isotype controls were used at  
23  
24  
25 141 a concentration of 1  $\mu$ L per 100  $\mu$ L of whole blood. Samples were measured using the  
26  
27 142 Gallios flow cytometer (Beckman Coulter, Brea, CA, USA) and analyzed using Gallios  
28  
29  
30 143 Software version 1.0 (Beckman Coulter). The flow cytometer was periodically  
31  
32  
33 144 calibrated by an engineer. Cells were stained for 20 min; thresholds were defined using  
34  
35 145 the manufacturer's recommended isotype controls. Representative plots and gating  
36  
37  
38 146 strategy from a single sample are shown in Supplemental Figure 2. T cells were gated  
39  
40 147 by CD3<sup>+</sup>CD4<sup>+</sup> or CD3<sup>+</sup>CD8<sup>+</sup>, B cells were gated by CD3<sup>-</sup>CD19<sup>+</sup>, NK cells were gated  
41  
42  
43 148 by CD16<sup>+</sup>CD56<sup>+</sup>, and Tregs were gated by CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>. At least 10,000  
44  
45  
46 149 events were collected in the lymphocyte cell gate for each sample. Results are expressed  
47  
48  
49 150 as percentages and mean fluorescence intensity (MFI) values.

50  
51 151 Absolute CD3<sup>+</sup> and CD4<sup>+</sup> lymphocyte, NK cell, and Treg cell counts were obtained  
52  
53 152 using Flow-Count fluorospheres (Beckman Coulter, Catalogue No. 7547053),  
54  
55  
56 153 according to the manufacturer's instructions. B, CD3<sup>+</sup>CD4<sup>+</sup>T, CD3<sup>+</sup>CD8<sup>+</sup>T, and Treg  
57  
58  
59 154 cell counts were calculated by their percentages in CD3<sup>+</sup> or CD4<sup>+</sup> lymphocytes  
60

1  
2  
3  
4 155 multiplied by CD3<sup>+</sup> or CD4<sup>+</sup> lymphocyte counts.  
5

6 156 The secondary outcomes of this study were plasma total cortisol and ACTH levels  
7  
8  
9 157 after ROSC. Venous blood samples were collected in heparin anticoagulant tubes,  
10  
11 158 centrifuged 10 min at 3000 rpm, and then stored at -80 °C. Plasma total cortisol  
12  
13 159 (IMMULITE 2000 Cortisol, L2KCO2, UK) and ACTH (IMMULITE 2000 ACTH,  
14  
15 160 L2KAC2, UK) levels were assayed using a chemiluminescent immunoassay on a  
16  
17 161 Siemens automated analyzer (IMMULITE 2000 XPi; Siemens Healthcare Diagnostics,  
18  
19 162 Erlangen, Germany). The equipment and reagents were calibrated by engineers before  
20  
21 163 use. The lower detection limit of total cortisol was 2.00 ng/mL, and that of ACTH was  
22  
23 164 5.00 pg/mL.  
24  
25  
26  
27  
28  
29

### 30 165 **Statistical analyses**

31  
32 166 Data analysis was used in SPSS version 22.0 (IBM Corp., Armonk, NY, USA) and  
33  
34 167 sample size calculation in PASS15.0 software (NCSS, LLC, Kaysville, UT, USA). For  
35  
36 168 normally distributed data, continuous variables are expressed as means with standard  
37  
38 169 deviations. Since the data for total cortisol and ACTH levels had a skewed distribution,  
39  
40 170 we compared our results with the natural logarithmic conversion values after adding 1  
41  
42 171 ( $\ln [\text{total cortisol} + 1]$ ,  $\ln [\text{ACTH} + 1]$ ). Measurement data with a skewed distribution are  
43  
44 172 expressed as medians (25th and 75th percentiles). The Mann–Whitney U test was used  
45  
46 173 to compare variables between groups. The qualitative parameters in the  $2 \times 2$   
47  
48 174 contingency table were used for analysis. All statistical tests were two-tailed, and a P-  
49  
50 175 value of  $<0.05$  was considered statistically significant.  
51  
52  
53  
54  
55  
56  
57

### 58 176 **Follow-up**

---

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  $\ln$  (total cortisol+1) and  $\ln$   
229 (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  $CD3^+CD4^+/T$ ,  
243  $CD3^+CD8^+/T$ , and Treg cell/ $CD4^+$  T lymphocyte ratios were significantly reduced after  
244 ROSC. NK cells, which are special innate immune cells with cytotoxic functions

1  
2  
3  
4 245 similar to CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes, mainly distinguish infected and stressed cells  
5  
6 246 from healthy cells and eliminate intracellular infection and dysfunctional cells. [29, 30]  
7  
8  
9 247 T lymphocytes are also crucial because they function as adaptive immune cells to  
10  
11 248 control and eliminate the infection. [29] Moreover, B and T lymphocytes mediate  
12  
13  
14 249 humoral and cellular immunity, respectively. This study was performed earlier and  
15  
16  
17 250 involved a more comprehensive assessment of the immune system of patients who  
18  
19 251 experienced CA. Our findings more substantially supported the rapid emergence of  
20  
21  
22 252 immune dysfunction in these patients after ROSC than in previous reports.  
23

24  
25 253 The binding of GCs to GR inside different peripheral blood mononuclear cells  
26  
27 254 (PBMC) leads to changes in the ability of cells to regulate apoptosis, proliferation, and  
28  
29  
30 255 activity, and GC-GR complexes limit the transcription (trans-repression) of  
31  
32  
33 256 inflammatory genes, including those encoding for proinflammatory cytokines.[31, 32]  
34  
35  
36 257 This study is the first to explore GR expression in circulating immune cells in patients  
37  
38 258 who experienced CA after ROSC. We observed that GR expression in B and T  
39  
40  
41 259 lymphocytes, NK cells, and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes decreased significantly in  
42  
43  
44 260 patients who experienced CA, whereas the percentage of GR<sup>+</sup> Treg cells and  
45  
46  
47 261 CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes decreased slightly. Moreover, we observed a more  
48  
49  
50 262 significant decrease in the MFI of GR expression in Treg cells and CD3<sup>+</sup>CD4<sup>+</sup> T  
51  
52  
53 263 lymphocytes but not in the percentage of GR expression. Previous studies have found  
54  
55  
56 264 decreased expression of GRs in peripheral polymorphonuclear cells in critically ill  
57  
58  
59 265 patients, [21] and antagonism to GRs aggravates viral and bacterial infections. [33]  
60  
266 GCs induced upon infections help to maintain homeostasis and mitigate the life-

1  
2  
3  
4 267 threatening impact of sepsis on the host.[31] Although studies have reported that the  
5  
6 268 use of GCs during and after CPR seems to confer benefits concerning ROSC rates and  
7  
8  
9 269 long-term survival, the evidence is scant. [13,18,34,35] Since cortisol signaling is  
10  
11 270 mediated by GRs, we hypothesized that the differential responses of CA patients to GC  
12  
13  
14 271 may be related to their levels of GR expression. This study suggests that the decrease  
15  
16  
17 272 in intracellular GR expression in patients who experienced CA is one of the causes of  
18  
19 273 GC resistance due to insufficient binding of GRs and GCs, GC insensitivity, and the  
20  
21  
22 274 inability of GCs to exert anti-inflammatory and immunosuppressive effects effectively.  
23  
24  
25 275 These findings may also explain why different results regarding the clinical application  
26  
27 276 of GCs have been reported previously. Furthermore, it is vital to measure GR levels as  
28  
29  
30 277 sufficient expression of GR is essential for mediating adequate GC effects during and  
31  
32  
33 278 after CPR.

34  
35 279 We also found that the total plasma cortisol levels were significantly higher in  
36  
37 280 patients who experienced CA, but ACTH levels were not. High levels of inflammatory  
38  
39  
40 281 cytokines inhibit ACTH release. [18] During critical illness, the body does not  
41  
42  
43 282 sufficiently metabolize cortisol. [36] In addition, the continuous increase in plasma  
44  
45  
46 283 cortisol levels may trigger the negative feedback pathway of the hypothalamic-  
47  
48 284 pituitary-adrenal axis, inhibiting the release of ACTH and cortisol and eventually  
49  
50  
51 285 leading to adrenal insufficiency [37]. These factors may explain the opposite trends of  
52  
53  
54 286 plasma ACTH and cortisol levels in the patients included in this study and who  
55  
56 287 experienced CA. Notably, this result suggests that low GR expression levels are not  
57  
58  
59 288 matched by high plasma total cortisol levels in patients who experienced CA. The  
60



1  
2  
3  
4 289 dissociation between low GR expression and high cortisol implies an abnormal stress  
5  
6 290 response. [38] Although systemic cortisol levels may be high, its availability is low  
7  
8  
9 291 during cardiac arrest. Previous studies have found that GC use during resuscitation may  
10  
11 292 benefit patients who experience CA. [13-16] Possible reasons for this response may be  
12  
13 293 that large doses of GCs given to CA patients may stimulate the function of GRs, or that  
14  
15 294 GR expression or GC sensitivity was better in some patients. The probability of  
16  
17 295 systemic inflammatory response and immunosuppression may also have been reduced  
18  
19 296 in some CA patients. This study did not provide data on plasma GC levels and GR  
20  
21 297 expression in a group of patients who were administered GCs and successfully  
22  
23 298 resuscitated; therefore, further studies are required.  
24  
25  
26  
27  
28  
29

30 299

### 31 32 **Limitations**

33  
34  
35 301 Our study has several limitations. First, to assess changes, we only enrolled patients  
36  
37 302 who experienced CA and had signs of systemic ischemic hypoxia, such as GCS <8 after  
38  
39 303 ROSC. The patients were not stratified by age, sex, the occurrence of comorbidities, or  
40  
41 304 mild systemic ischemic hypoxia. Second, since this was a preliminary observational  
42  
43 305 study, we observed only early changes. A more relevant control group and dynamic  
44  
45 306 observations obtained over a longer duration would be helpful to understand the  
46  
47 307 significance of GR expression in evolving immunity during the clinical course of CA  
48  
49 308 after ROSC. Third, the samples used in this study were from clinical laboratories; thus,  
50  
51 309 plasma total cortisol and ACTH in the samples were at risk of degradation before we  
52  
53 310 collected the samples. Finally, we did not discuss the changes in and roles of GR  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4 311 isoforms, free cortisol, and corticosteroid-binding globulin. Therefore, future studies  
5  
6 312 on these aspects are warranted to better understand the immunosuppressive effects of  
7  
8  
9 313 ROSC among patients who experienced CA.

10  
11 314 In conclusion, this study revealed that GR expression, cell counts and ratios rapidly  
12  
13  
14 315 decreased, whereas plasma total cortisol levels increased, in the early period after  
15  
16  
17 316 ROSC among CA patients. These findings may provide important information about  
18  
19  
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  
23  
24  
25 319 sensitive to glucocorticoid therapy.

26  
27 320  
28  
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  
37 324 **Contributorship statement:** CL designed the study and reviewed the manuscript.  
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.

49  
50 329 **Competing interests:** All authors declare no competing interest associated with this  
51  
52  
53 330 project.

54  
55  
56 331 **Funding:** This research received no specific grant from any funding agency in public,  
57  
58 332 commercial or not-for-profit sectors.

---

1  
2  
3  
4 333 **Provenance and peer review:** Not commissioned; externally peer-reviewed.  
5

6 334 **Data sharing statement:** All data relevant to the study are included in the article or  
7  
8  
9 335 uploaded as supplementary information. Due to privacy and ethical concerns, data can  
10  
11  
12 336 not be shared.  
13

14 337

15  
16  
17 338 **Ethics statements**

18  
19 339 **Patient consent for publication:** Not applicable.  
20

21  
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  
26  
27 342 obtained from the families of the patients to enroll them in the study.  
28  
29

30 343  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

---

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

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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

- 
- 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

- 
- 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  
29 464 DOI: 10.1016/j.mce.2019.110656.  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

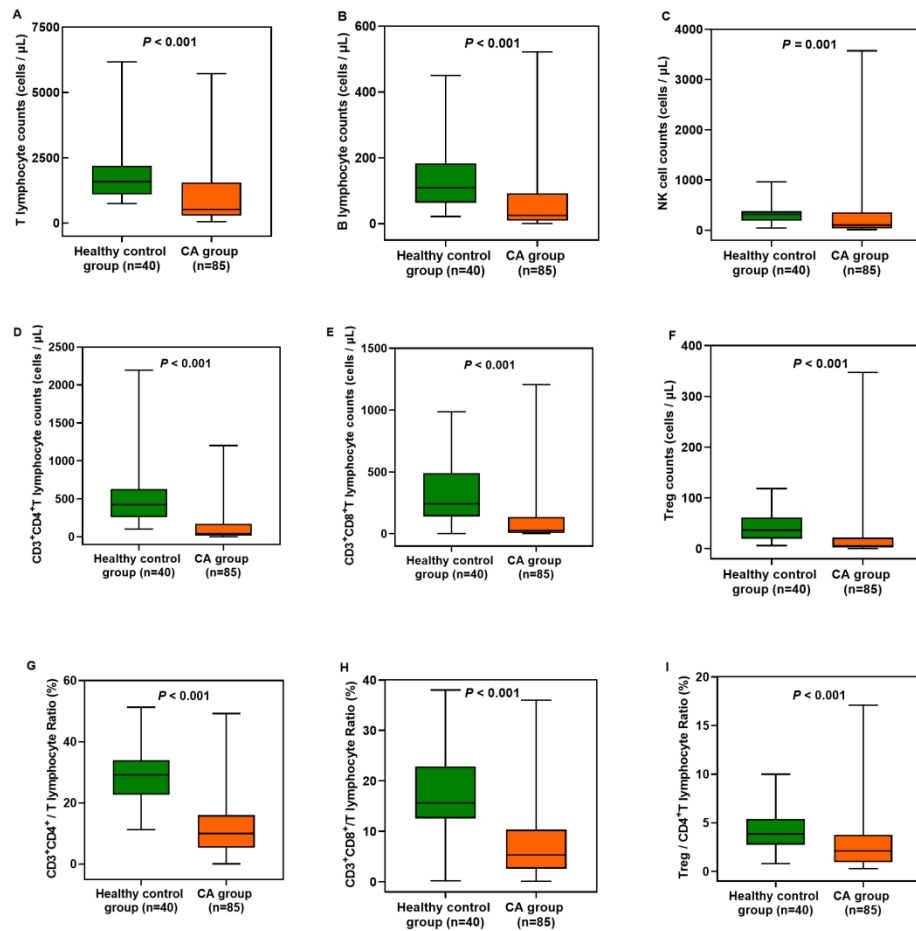
---

1  
2  
3  
4 465 **Figure legends**

5  
6 466 **Fig. 1.** Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts,  
7  
8  
9 467 CD3<sup>+</sup>CD4<sup>+</sup>/T, CD3<sup>+</sup>CD8<sup>+</sup>/T, and Treg/CD4<sup>+</sup>T lymphocyte ratios between the healthy  
10  
11 468 control group and CA group. The CA group showed significant differences compared  
12  
13  
14 469 with the healthy control group (P<0.001). CA, cardiac arrest; CD, cluster-of-  
15  
16  
17 470 differentiation; NK, natural killer; Treg, regulatory T.

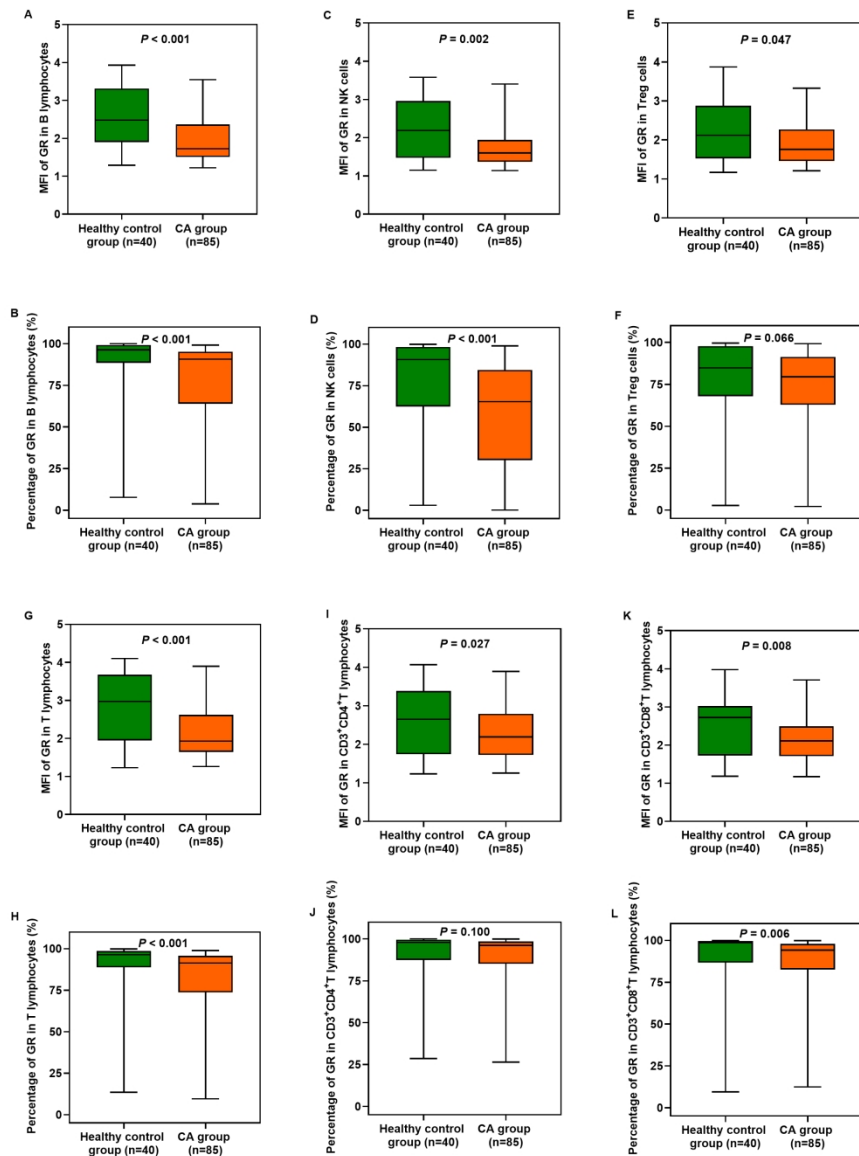
18  
19 471 **Fig. 2.** Expression of GRs in circulatory T and B lymphocytes, NK cells, and Treg cells  
20  
21  
22 472 in the healthy control group and CA group. The CA group showed significant  
23  
24 473 differences compared with the healthy control group (P<0.05). CA, cardiac arrest; CD,  
25  
26  
27 474 cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC,  
28  
29  
30 475 return of spontaneous circulation; Treg, regulatory T.

31  
32 476 **Fig. 3.** (A, B) Plasma total cortisol and ACTH levels (the natural logarithmic  
33  
34  
35 477 conversion values after adding 1) after ROSC in the healthy control group and CA  
36  
37  
38 478 group. (C, D) Plasma total cortisol and ACTH levels in survivors and non-survivors  
39  
40  
41 479 after ROSC. The CA group showed significant differences compared with the healthy  
42  
43  
44 480 control group (P<0.05). ACTH, adrenocorticotrophic hormone; CA, cardiac arrest;  
45  
46  
47 481 ROSC, return of spontaneous circulation.



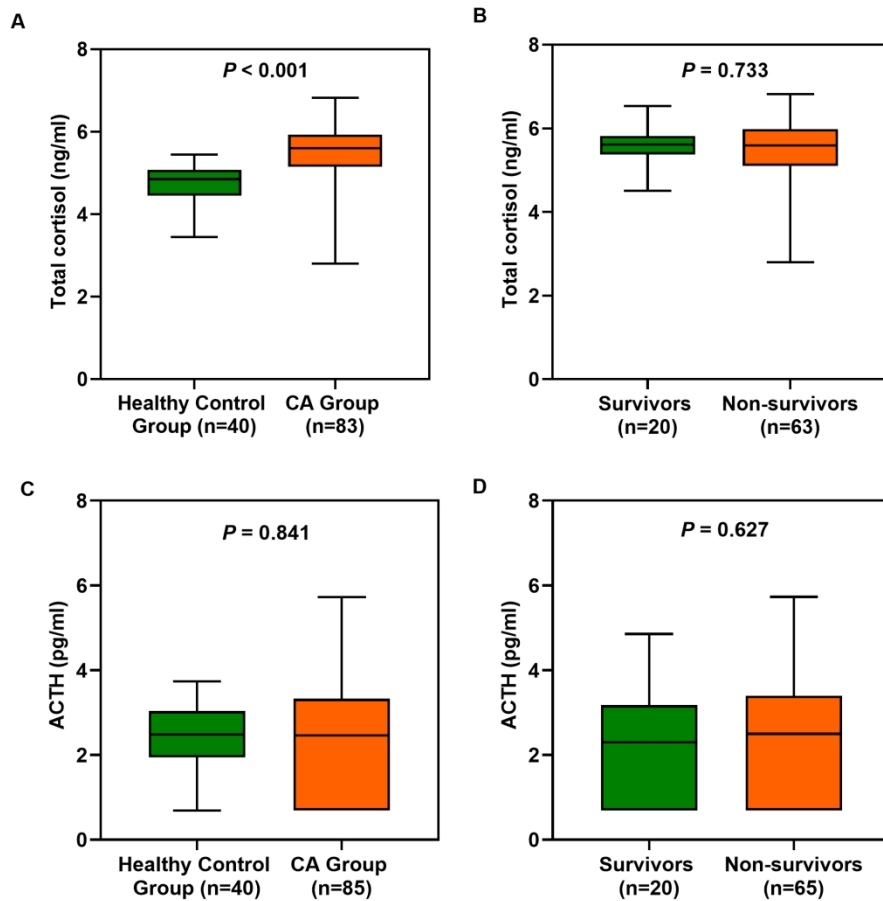
Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and CD3+CD4+/T, CD3+CD8+/T, and Treg/CD4+T lymphocyte ratios between the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group ( $P < 0.001$ ). CA, cardiac arrest;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

Yanan Yu<sup>1</sup>; Ziren Tang<sup>1</sup>; Miaorong Xie<sup>2</sup>; Jiabao Li<sup>3</sup>; Chenchen Hang<sup>1</sup>; Le An<sup>1</sup>; Chunsheng Li<sup>1, \*</sup>

<sup>1</sup>Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

<sup>2</sup>Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing 100032, China

<sup>3</sup>Department of Critical Care, Beijing Friendship Hospital, Capital Medical University, Beijing 100020, China

\*Corresponding author: Chungsheng Li, M.D.

Department of Emergency Medicine, Beijing Chaoyang Hospital,

Capital Medical University, 8 Worker's Stadium South Road, Chaoyang District, Beijing 100020, China, Tel: [+86](tel:+8613681392380)

[13681392380](tel:+8613681392380); E-mail: [lcscy@163.com](mailto:lcscy@163.com)

#### Contents

Supplemental Figure 1

Supplemental Figure 2

Supplemental Table 1

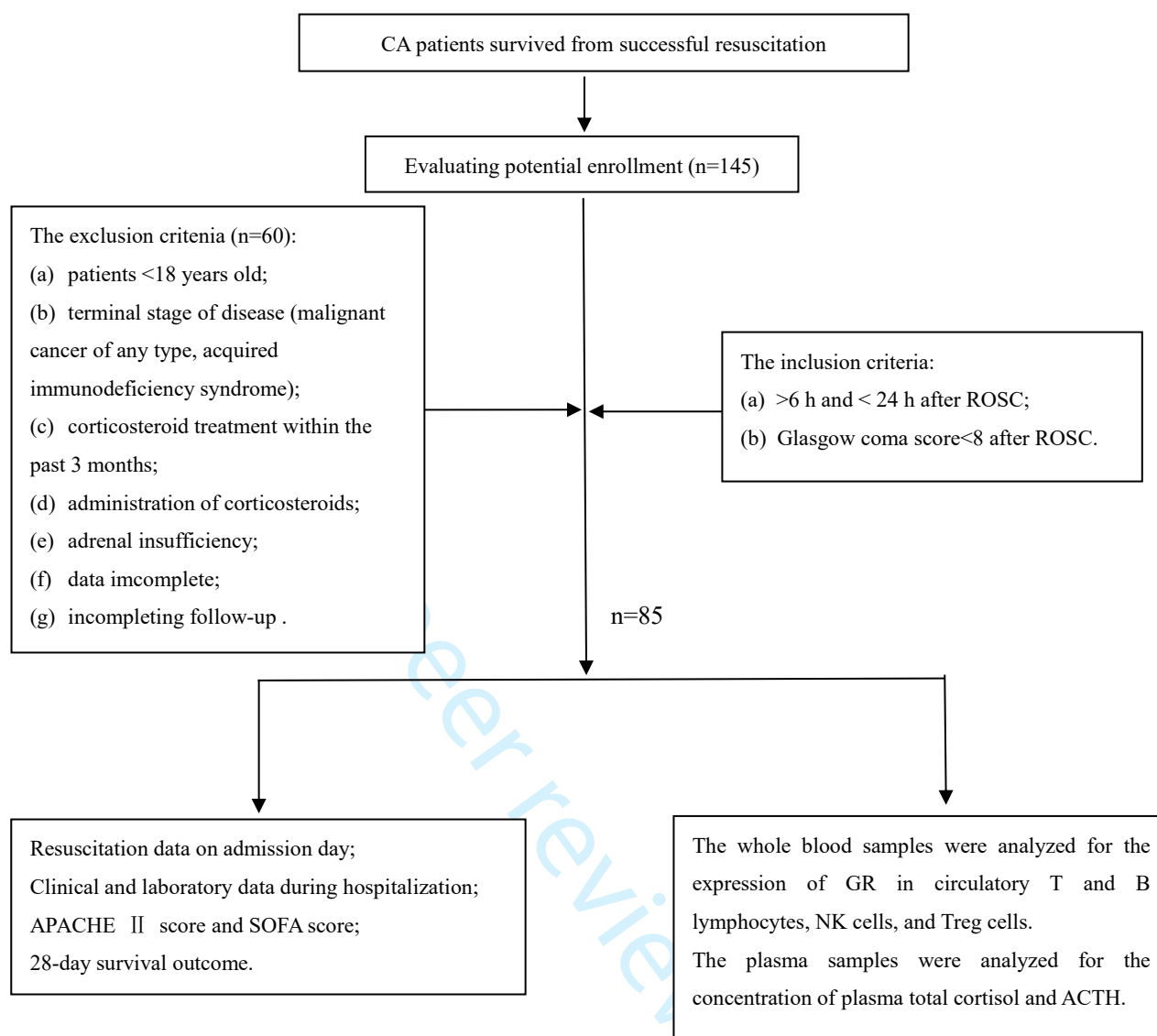
Supplemental Table 2

Supplemental Table 3

Supplemental Table 4

Supplemental Table 5

Supplemental Table 6



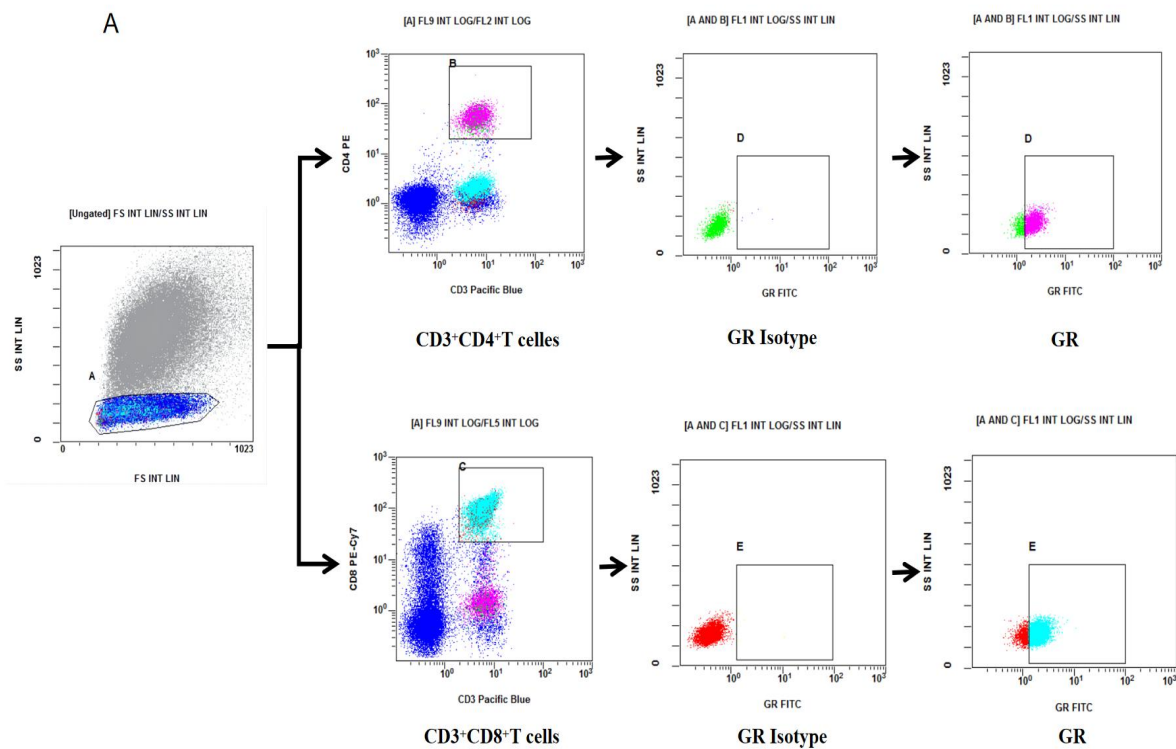
Supplemental Figure 1. The flow chart of the study.

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<sup>-</sup>CD19<sup>+</sup> cells. NK cells were identified as CD16<sup>+</sup>56<sup>+</sup> cells. T cells were identified as CD3<sup>+</sup>CD4<sup>+</sup> T cells and CD3<sup>+</sup>CD8<sup>+</sup> T cells. Treg cells were identified as CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>.

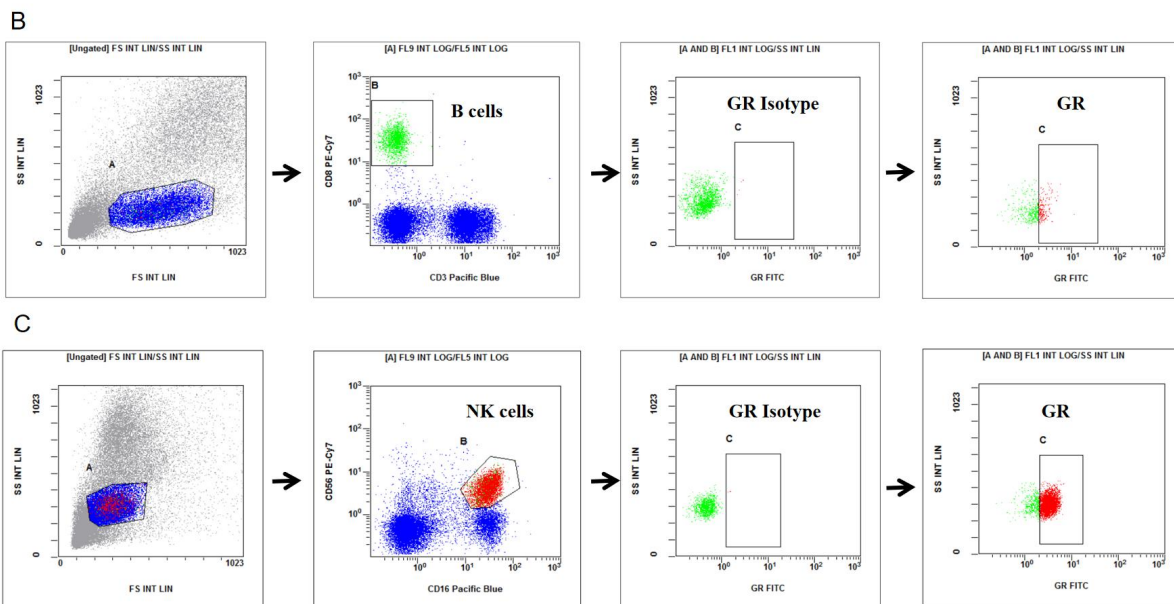
#### A. Expression of GR on T cells



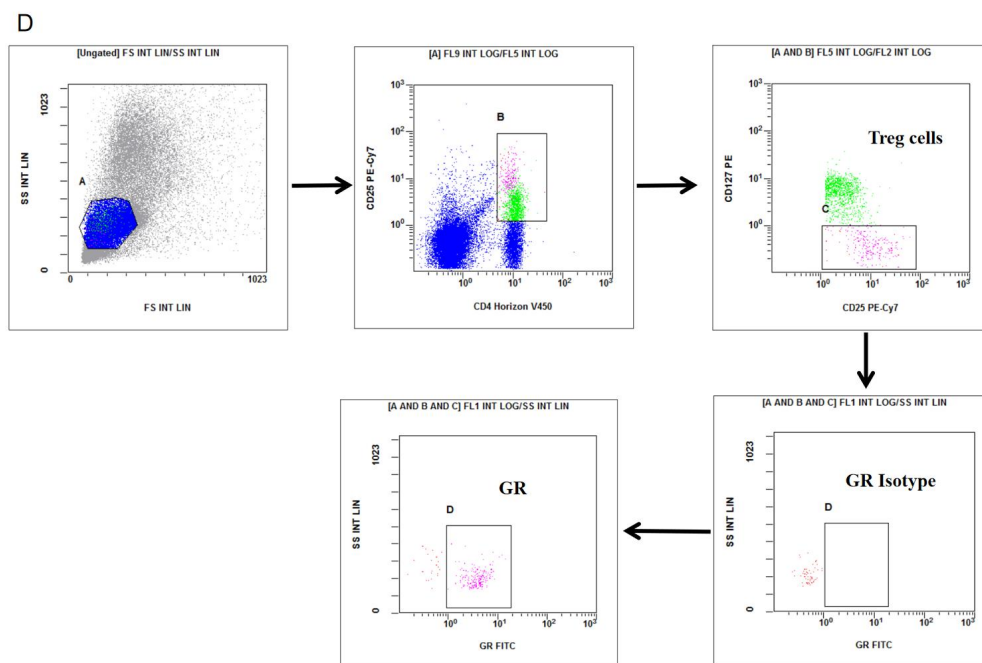


B. Expression of GR on B cells

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 <sup>a</sup>
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 <sup>b</sup>
Mouse IgG1 Isotype	MCA928F	FITC	Bio-Rad
Mouse IgG1, $\kappa$ Isotype	557872	PE-Cy7	BD Pharmingen
Mouse IgG1, $\kappa$ Isotype	554680	PE	BD Pharmingen
Mouse IgG1, $\kappa$ Isotype	558120	Pacific Blue	BD Pharmingen

<sup>a</sup> BD Pharmingen, San Diego, USA; <sup>b</sup> 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 / $\mu$ L)	1586.0 (1101.5, 2192.5)	514.0 (287.5, 1555.0)	-4.515	<0.001
NK cell count (/ $\mu$ L)	311.5 (191.0, 378.8)	101.0 (36.0, 351.5)	-3.332	0.001
B lymphocyte count (/ $\mu$ L)	109.3 (63.7, 183.3)	25.7 (9.4, 92.3)	-5.076	<0.001
Treg count (/ $\mu$ L)	0.259 (0.095, 0.516)	0.233 (0.135, 0.488)	-5.518	<0.001
Treg / CD4 <sup>+</sup> T lymphocyte Ratio	0.039 (0.028, 0.054)	0.021 (0.010, 0.038)	-4.418	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocyte count (/ $\mu$ L)	421.7 (258.6, 627.4)	38.9 (17.6, 168.3)	-6.256	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup> / T lymphocyte Ratio	0.292 (0.227, 0.340)	0.100 (0.054, 0.160)	-7.066	<0.001
CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocyte count (/ $\mu$ L)	241.1 (139.5, 488.6)	26.3 (7.2, 135.9)	-5.287	<0.001
CD3 <sup>+</sup> CD8 <sup>+</sup> / 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 <sup>+</sup> T lymphocyte Ratio	0.025 (0.009, 0.043)	0.021 (0.010, 0.034)	-0.498	0.619
CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocyte count (/μL)	55.1 (32.4, 228.0)	38.0 (16.0, 168.1)	-0.850	0.396
CD3 <sup>+</sup> CD4 <sup>+</sup> / T lymphocyte Ratio	0.118 (0.070, 0.236)	0.097 (0.049, 0.142)	-1.565	0.118
CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocyte count (/μL)	25.4 (12.5, 96.2)	26.3 (6.3, 138.8)	-0.021	0.983
CD3 <sup>+</sup> CD8 <sup>+</sup> / 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 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	0.980 (0.874, 0.996)	0.957 (0.824, 0.985)	-2.204	0.100
MFI of GR on CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	2.65 (1.75, 3.38)	2.17 (1.70, 2.92)	-1.646	0.027
Percentage of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocytes	0.986 (0.868, 0.996)	0.938 (0.823, 0.979)	-2.758	0.006
MFI of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> 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 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	0.975 (0.876, 0.985)	0.957 (0.845, 0.987)	-0.617	0.538
MFI of GR on CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	2.08 (1.72, 3.35)	2.22 (1.71, 2.69)	-0.865	0.387
Percentage of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocytes	0.963 (0.816, 0.977)	0.938 (0.834, 0.980)	-0.254	0.800
MFI of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> 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
<b>Title and abstract</b>	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
<b>Introduction</b>			
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
<b>Methods</b>			
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	

Continued on next page

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
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
---------	----	---	----

\*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](http://www.strobe-statement.org).  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

# BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-060246.R2
Article Type:	Original research
Date Submitted by the Author:	10-Aug-2022
Complete List of Authors:	Yu, Yanan; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine Tang, Ziren; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine Xie, Miaorong; Capital Medical University Affiliated Beijing Friendship Hospital, Department of Emergency Medicine Li, Jiabao; Capital Medical University Affiliated Beijing Friendship Hospital, Department of Critical Care Hang, Chen-Chen; Beijing Chao-Yang Hospital, Emergency Medicine An, Le; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine Li, Chunsheng; Beijing Chao-Yang Hospital Capital Medical University, Department of Emergency Medicine
<b>Primary Subject Heading</b>:	Emergency medicine
Secondary Subject Heading:	Intensive care
Keywords:	ACCIDENT & EMERGENCY MEDICINE, INTENSIVE & CRITICAL CARE, Adult intensive & critical care < INTENSIVE & CRITICAL CARE

SCHOLARONE™  
Manuscripts



I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our [licence](#).

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which [Creative Commons](#) licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

---

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**

4 Yanan Yu<sup>1</sup>; Ziren Tang<sup>1</sup>; Miaorong Xie<sup>2</sup>; Jiabao Li<sup>3</sup>; Chenchen Hang<sup>1</sup>; Le An<sup>1</sup>;  
5 Chunsheng Li<sup>1</sup>,\*

6 <sup>1</sup>Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical  
7 University, Beijing 100020, China

8 <sup>2</sup>Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical  
9 University, Beijing 100032, China

10 <sup>3</sup>Department of Critical Care, Beijing Friendship Hospital, Capital Medical University,  
11 Beijing 100020, China

12 \*Corresponding author: Chungsheng Li, M.D.

13 Department of Emergency Medicine, Beijing Chaoyang Hospital,  
14 Capital Medical University, 8 Worker's Stadium South Road, Chaoyang District,  
15 Beijing 100020, China, Tel: [+86 13681392380](tel:+8613681392380); E-mail: [lcseyyy@163.com](mailto:lcseyyy@163.com)

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-

1  
2  
3  
4 45 survivors.

5  
6 46 **Conclusions:** This study revealed that GR expression and cell counts rapidly decreased,  
7  
8  
9 47 whereas plasma total cortisol levels increased in the early period after ROSC among  
10  
11  
12 48 patients who experienced CA. Our findings provide important information about GR  
13  
14  
15 49 level and function, and immunosuppressive status in these patients. Assessing GR  
16  
17  
18 50 expression in CA patients may help screening for those who are more sensitive to  
19  
20 51 glucocorticoid therapy.  
21

22 52

### 23 24 25 53 **Strengths and limitations of this study**

- 26  
27 54 1. The study was designed as single-center, prospective study.  
28  
29  
30 55 2. This is the first study to evaluate the GR expression in the early period following  
31  
32  
33 56 ROSC among CA patients.  
34  
35 57 3. We only studied the GR expression of CA patients in the early period following  
36  
37  
38 58 ROSC; therefore, our results cannot be extrapolated to time points beyond 24 hours.  
39  
40 59 4. Decreased GR expression may affect the sensitivity of CA patients to GCs.  
41  
42  
43 60 5. Decreased GR expression may affect potential immune consequences of CA  
44  
45 61 patients.  
46

47  
48 62

### 49 50 51 63 **Introduction**

52  
53 64 Cardiac arrest (CA) is a significant health problem globally; about 356,500 people  
54  
55  
56 65 experience medical emergencies due to CA in the United States, and over 544,000  
57  
58  
59 66 people die from sudden CA in China annually. [1, 2] The systemic ischemia-reperfusion  
60



1  
2  
3  
4 67 response in patients who have experienced CA can present as post-cardiac arrest  
5  
6 68 syndrome (PCAS) or systematic inflammatory response syndrome (SIRS), which  
7  
8  
9 69 increases the risk of multiple organ failure and infection and affects the inflammatory  
10  
11  
12 70 response and prognosis of patients after the return of spontaneous circulation (ROSC).  
13  
14 71 [3-6]

17 72 CA is the most intense among acute stress events, which seriously affect the pituitary  
18  
19 73 and adrenal axis function. [7] Studies have shown that abnormal cortisol levels and  
20  
21  
22 74 relative adrenocortical insufficiency after ROSC in patients who experienced CA are  
23  
24  
25 75 related to their prognosis. [8-11] However, the clinical application of glucocorticoids  
26  
27 76 (GCs) is controversial. In the 2015 International Cardiopulmonary Resuscitation  
28  
29  
30 77 Guidelines, the routine use of GCs is not recommended for the resuscitation of patients  
31  
32  
33 78 with in-hospital or out-of-hospital CA. [12] Recent clinical studies have shown that  
34  
35 79 early administration of corticosteroids after CA can improve the success rate of ROSC,  
36  
37  
38 80 nervous system functional outcome, and prognosis, which is speculated to be related to  
39  
40  
41 81 its influence on hemodynamics, and SIRS response, and other mechanisms. [12-17]  
42  
43 82 Therefore, the role of GCs in the occurrence and development of PCAS needs to be  
44  
45  
46 83 studied further.

48 84 GCs combine with intracellular GC receptors (GRs) to exert anti-inflammatory and  
49  
50  
51 85 immunosuppressive effects and reduce the production and the release of inflammatory  
52  
53  
54 86 cytokines. [18, 19] The affinity of GRs to GCs in circulating monocytes is decreased in  
55  
56  
57 87 patients with acquired immunodeficiency syndrome. [20] The expression of GR alpha  
58  
59 88 and beta in peripheral polymorphonuclear cells is decreased in patients with critical  
60

1  
2  
3  
4 89 illness, [21] pediatric septic shock, and high serum cortisol levels. [22] However, no  
5  
6 90 study has reported the GR expression after ROSC in patients who experienced CA.  
7  
8  
9 91 Previous studies have found that the counts of circulating B and T lymphocytes,  
10  
11 92 regulatory T (Treg) cells, and monocytes and expression of human leukocyte antigen  
12  
13 93 DR (HLA-DR) on circulatory monocytes and B and T lymphocytes are reduced. [23,  
14  
15 94 24] Hence, this study aimed to investigate the relationship between GR expression and  
16  
17 95 immune alteration in the early period after ROSC in patients who experienced CA by  
18  
19 96 observing GR expression in circulatory T and B lymphocytes, NK cells, and Treg cells,  
20  
21 97 their cell counts, and total plasma cortisol and adrenocorticotrophic hormone (ACTH)  
22  
23 98 levels.  
24  
25  
26  
27  
28  
29  
30  
31  
32

## 33 **Materials and methods**

### 34 **Study participants**

35  
36  
37 102 This was an observational study conducted in the Emergency Department (ED).  
38  
39 103 According to the 2015 International Cardiopulmonary Resuscitation Guidelines, [25]  
40  
41 104 we enrolled patients in the early ROSC period after CA (both in-hospital and out-of-  
42  
43 105 hospital CA) and were admitted to the ED between October 2018 and October 2019.  
44  
45  
46  
47 106 The inclusion criteria were patients with CA > 6 and < 24 hours after ROSC, with a  
48  
49 107 Glasgow coma score < 8. The exclusion criteria were (a) <18 years of age, (b) terminal  
50  
51 108 stage of disease (such as cancer of any type, acquired immunodeficiency syndrome),  
52  
53 109 (c) corticosteroid treatment within the past three months, (d) administration of  
54  
55 110 corticosteroids, and (e) adrenal insufficiency. All patients were treated according to the  
56  
57  
58  
59  
60

---

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- $\mu$ L peripheral blood

1  
2  
3  
4 133 sample was stained for 20 min with surface antibodies (CD3, CD4, CD8, CD19, CD16,  
5  
6 134 CD56, CD25, and CD127) in a dark place. Erythrocytes were lysed for 15 min, and the  
7  
8  
9 135 debris was washed away. Before staining of the intracellular GR antibody and its  
10  
11 136 isotype control (Bio-Rad AbD Serotec, Oxford, UK), surface-stained cells were fixed  
12  
13  
14 137 and permeabilized using the BD Transcription Factor Buffer Set (BD Pharmingen, San  
15  
16  
17 138 Diego, USA, Catalogue No. 562574). Monoclonal antibodies and their isotype controls  
18  
19 139 were all purchased from BD Biosciences (San Jose, CA, USA). Details of all antibodies  
20  
21  
22 140 are shown in Supplemental Table 1. According to the manufacturer's recommendations,  
23  
24  
25 141 all antibodies and their isotype controls were used at a concentration of 1  $\mu$ L per 100  
26  
27 142  $\mu$ L of whole blood. Samples were measured using the Gallios flow cytometer (Beckman  
28  
29  
30 143 Coulter, Brea, CA, USA) and analyzed using Gallios Software version 1.0 (Beckman  
31  
32  
33 144 Coulter). The flow cytometer was periodically calibrated by an engineer. Cells were  
34  
35 145 stained for 20 min; thresholds were defined using the manufacturer's recommended  
36  
37  
38 146 isotype controls. Representative plots and gating strategy from a single sample are  
39  
40 147 shown in Supplemental Figure 2. T cells were gated by CD3<sup>+</sup>CD4<sup>+</sup> or CD3<sup>+</sup>CD8<sup>+</sup>, B  
41  
42  
43 148 cells were gated by CD3<sup>-</sup>CD19<sup>+</sup>, NK cells were gated by CD16<sup>+</sup>CD56<sup>+</sup>, and Tregs were  
44  
45  
46 149 gated by CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low</sup>. At least 10,000 events were collected in the  
47  
48  
49 150 lymphocyte cell gate for each sample. Results are expressed as percentages and mean  
50  
51  
52 151 fluorescence intensity (MFI) values.

53 152 Absolute CD3<sup>+</sup> and CD4<sup>+</sup> lymphocyte, NK cell, and Treg cell counts were obtained  
54  
55  
56 153 using Flow-Count fluorospheres (Beckman Coulter, Catalogue No. 7547053),  
57  
58  
59 154 according to the manufacturer's instructions. B, CD3<sup>+</sup>CD4<sup>+</sup>T, CD3<sup>+</sup>CD8<sup>+</sup>T, and Treg

1  
2  
3  
4 155 cell counts were calculated by their percentages in CD3<sup>+</sup> or CD4<sup>+</sup> lymphocytes  
5  
6 156 multiplied by CD3<sup>+</sup> or CD4<sup>+</sup> lymphocyte counts.  
7  
8

9 157 The secondary outcomes of this study were plasma total cortisol and ACTH levels  
10  
11 158 after ROSC. Venous blood samples were collected in heparin anticoagulant tubes,  
12  
13 159 centrifuged 10 min at 3000 rpm, and then stored at -80 °C. Plasma total cortisol  
14  
15 160 (IMMULITE 2000 Cortisol, L2KCO2, UK) and ACTH (IMMULITE 2000 ACTH,  
16  
17 161 L2KAC2, UK) levels were assayed using a chemiluminescent immunoassay on a  
18  
19 162 Siemens automated analyzer (IMMULITE 2000 XPi; Siemens Healthcare Diagnostics,  
20  
21 163 Erlangen, Germany). The equipment and reagents were calibrated by engineers before  
22  
23 164 use. The lower detection limit of total cortisol was 2.00 ng/mL, and that of ACTH was  
24  
25 165 5.00 pg/mL.  
26  
27  
28  
29  
30  
31

### 32 166 **Sample size calculation and statistical analysis**

33  
34  
35 167 The sample size was calculated using the PASS15.0 software (NCSS, LLC,  
36  
37 168 Kaysville, UT, USA) and the non-parametric test method. The median GR expression  
38  
39 169 was 0.93 and 0.80 in the healthy and CA groups, respectively, and the interquartile  
40  
41 170 spacing was 0.1 and 0.3. According to the ratio of 1:2 between the two groups, with a  
42  
43 171 test level of 0.05 and a confidence interval of 0.90, a total of 105 samples were required,  
44  
45 172 comprising at least 35 in the healthy group and 70 in the CA group. The number of  
46  
47 173 people included in the two groups in this study was 40 and 85, respectively, which met  
48  
49 174 our research requirements. Data analysis was used in SPSS version 22.0 (IBM Corp.,  
50  
51 175 Armonk, NY, USA). For normally distributed data, continuous variables are expressed  
52  
53 176 as means with standard deviations. Since the data for total cortisol and ACTH levels  
54  
55  
56  
57  
58  
59  
60

---

177 had a skewed distribution, we compared our results with the natural logarithmic  
178 conversion values after adding 1 ( $\ln [\text{total cortisol} + 1]$ ,  $\ln [\text{ACTH} + 1]$ ). Measurement  
179 data with a skewed distribution are expressed as medians (25th and 75th percentiles).  
180 The Mann–Whitney U test was used to compare variables between groups. The  
181 qualitative parameters in the  $2 \times 2$  contingency table were used for analysis. All  
182 statistical tests were two-tailed, and a P-value of  $<0.05$  was considered statistically  
183 significant.

#### 184 **Follow-up**

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

#### 188 **Patient and public involvement**

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

### 192 **Results**

#### 193 **Patient characteristics**

194 40 healthy individuals and 85 patients who experienced CA were analyzed. The  
195 demographics and clinical characteristics of both groups are shown in Table 1. In this  
196 study, acute cardiac and brain events were the main causes of CA, with those in the  
197 latter category emanating from strokes. Other causes of CA included poisoning  
198 (including carbon monoxide poisoning) and hypokalemia. Sex and age were not

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



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

### Changes in plasma total cortisol and ACTH levels after ROSC

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

### Discussion

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

1  
2  
3  
4 246 the controls.

5  
6 247 The ischemia-reperfusion response initiates an acute inflammatory response that  
7  
8  
9 248 contributes to post-resuscitation shock after CA.[27] The immune response of patients  
10  
11  
12 249 who experience CA is impaired, and the systemic inflammatory response increases. [6,  
13  
14 250 28] The T and B lymphocyte, NK cell, and Treg cell counts and CD3<sup>+</sup>CD4<sup>+</sup>/T,  
15  
16 251 CD3<sup>+</sup>CD8<sup>+</sup>/T, and Treg cell/CD4<sup>+</sup> T lymphocyte ratios were significantly reduced after  
17  
18  
19 252 ROSC. NK cells, which are special innate immune cells with cytotoxic functions  
20  
21  
22 253 similar to CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes, mainly distinguish infected and stressed cells  
23  
24  
25 254 from healthy cells and eliminate intracellular infection and dysfunctional cells. [29, 30]  
26  
27  
28 255 T lymphocytes are also crucial because they function as adaptive immune cells to  
29  
30  
31 256 control and eliminate the infection. [29] Moreover, B and T lymphocytes mediate  
32  
33  
34 257 humoral and cellular immunity, respectively. This study was performed earlier and  
35  
36  
37 258 involved a more comprehensive assessment of the immune system of patients who  
38  
39  
40 259 experienced CA. Our findings more substantially supported the rapid emergence of  
41  
42  
43 260 immune dysfunction in these patients after ROSC than in previous reports.

44  
45 261 The binding of GCs to GR inside different peripheral blood mononuclear cells  
46  
47  
48 262 (PBMC) leads to changes in the ability of cells to regulate apoptosis, proliferation, and  
49  
50  
51 263 activity, and GC-GR complexes limit the transcription (trans-repression) of  
52  
53  
54 264 inflammatory genes, including those encoding for proinflammatory cytokines.[31, 32]  
55  
56  
57 265 This study is the first to explore GR expression in circulating immune cells in patients  
58  
59  
60 266 who experienced CA after ROSC. We observed that GR expression in B and T  
267 lymphocytes, NK cells, and CD3<sup>+</sup>CD8<sup>+</sup> T lymphocytes decreased significantly in

1  
2  
3  
4 268 patients who experienced CA, whereas the percentage of GR<sup>+</sup> Treg cells and  
5  
6 269 CD3<sup>+</sup>CD4<sup>+</sup> T lymphocytes decreased slightly. Moreover, we observed a more  
7  
8  
9 270 significant decrease in the MFI of GR expression in Treg cells and CD3<sup>+</sup>CD4<sup>+</sup> T  
10  
11 271 lymphocytes but not in the percentage of GR expression. Previous studies have found  
12  
13  
14 272 decreased expression of GRs in peripheral polymorphonuclear cells in critically ill  
15  
16  
17 273 patients, [21] and antagonism to GRs aggravates viral and bacterial infections. [33]  
18  
19 274 GCs induced upon infections help to maintain homeostasis and mitigate the life-  
20  
21  
22 275 threatening impact of sepsis on the host.[31] Although studies have reported that the  
23  
24  
25 276 use of GCs during and after CPR seems to confer benefits concerning ROSC rates and  
26  
27  
28 277 long-term survival, the evidence is scant. [13,18,34,35] Since cortisol signaling is  
29  
30  
31 278 mediated by GRs, we hypothesized that the differential responses of CA patients to GC  
32  
33  
34 279 may be related to their levels of GR expression. This study suggests that the decrease  
35  
36  
37 280 in intracellular GR expression in patients who experienced CA is one of the causes of  
38  
39  
40 281 GC resistance due to insufficient binding of GRs and GCs, GC insensitivity, and the  
41  
42  
43 282 inability of GCs to exert anti-inflammatory and immunosuppressive effects effectively.  
44  
45  
46 283 These findings may also explain why different results regarding the clinical application  
47  
48  
49 284 of GCs have been reported previously. Furthermore, it is vital to measure GR levels as  
50  
51  
52 285 sufficient expression of GR is essential for mediating adequate GC effects during and  
53  
54  
55 286 after CPR.

56  
57  
58 287 We also found that the total plasma cortisol levels were significantly higher in  
59  
60 288 patients who experienced CA, but ACTH levels were not. High levels of inflammatory  
289 289 cytokines inhibit ACTH release. [18] During critical illness, the body does not

---

1  
2  
3  
4 290 sufficiently metabolize cortisol. [36] In addition, the continuous increase in plasma  
5  
6 291 cortisol levels may trigger the negative feedback pathway of the hypothalamic-  
7  
8  
9 292 pituitary-adrenal axis, inhibiting the release of ACTH and cortisol and eventually  
10  
11 293 leading to adrenal insufficiency [37]. These factors may explain the opposite trends of  
12  
13  
14 294 plasma ACTH and cortisol levels in the patients included in this study and who  
15  
16  
17 295 experienced CA. Notably, this result suggests that low GR expression levels are not  
18  
19  
20 296 matched by high plasma total cortisol levels in patients who experienced CA. The  
21  
22 297 dissociation between low GR expression and high cortisol implies an abnormal stress  
23  
24  
25 298 response. [38] Previous studies have reported that GR-action was clearly suppressed  
26  
27 299 throughout critical illness; GR resistance could not be overcome by further increasing  
28  
29  
30 300 glucocorticoid availability.[21,39,40] Adequate GR levels and function are also  
31  
32 301 required for normal GC function, which may explain differences in the responsiveness  
33  
34  
35 302 of cardiac arrest patients to exogenous steroid administration or endogenous cortisol  
36  
37  
38 303 secretion. Thus, actual GR levels cannot be reflected by measuring total cortisol levels  
39  
40  
41 304 alone. Therefore, the GR level should be considered when applying personalized GC  
42  
43  
44 305 therapy. The determination of GR expression might help to screen those who might  
45  
46 306 respond better to glucocorticoid prescription.

47  
48  
49 307

### 50 308 **Limitations**

51  
52  
53 309 Our study has several limitations. First, to assess changes, we only enrolled patients  
54  
55  
56 310 who experienced CA and had signs of systemic ischemic hypoxia, such as GCS <8 after  
57  
58  
59 311 ROSC. The patients were not stratified by age, sex, the occurrence of comorbidities, or

---

1  
2  
3  
4 312 mild systemic ischemic hypoxia. Second, since this was a preliminary observational  
5  
6 313 study, we observed only early changes. A more relevant control group and dynamic  
7  
8  
9 314 observations obtained over a longer duration would be helpful to understand the  
10  
11 315 significance of GR expression in evolving immunity during the clinical course of CA  
12  
13  
14 316 after ROSC. Third, the samples used in this study were from clinical laboratories; thus,  
15  
16  
17 317 plasma total cortisol and ACTH in the samples were at risk of degradation before we  
18  
19 318 collected the samples. Finally, we did not discuss the changes in and roles of GR  
20  
21 319 isoforms, free cortisol, and corticosteroid-binding globulin. Therefore, future studies  
22  
23 320 on these aspects are warranted to better understand the immunosuppressive effects of  
24  
25 321 ROSC among patients who experienced CA.  
26  
27  
28  
29

30 322 In conclusion, this study revealed that GR expression, cell counts and ratios rapidly  
31  
32 323 decreased, whereas plasma total cortisol levels increased, in the early period after  
33  
34 324 ROSC among CA patients. These findings may provide important information about  
35  
36 325 GR expression levels and function, and immunosuppressive status in these patients.  
37  
38  
39

40 326  
41  
42  
43 327 **Acknowledgments:** We thank all the patients and their families who were enrolled in  
44  
45 328 this study and colleagues from the emergency department who provided support. And  
46  
47 329 we are grateful for the efforts of the staff for ongoing resuscitation in hospitals.  
48  
49

50 330 **Contributorship statement:** CL designed the study and reviewed the manuscript.  
51  
52 331 YNY searched the literature and contributed to the experimental studies, data analysis,  
53  
54 332 and manuscript writing. ZRT, CCH, and LA collected and analyzed data. JBL and MRX  
55  
56  
57  
58  
59  
60

---

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.

337 **Funding:** This research received no specific grant from any funding agency in public,  
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.

350

---

**References**

- [1] Myat A, Song KJ, Rea T. Out-of-hospital cardiac arrest: current concepts. *Lancet*, Mar 10, 2018. DOI: 10.1016/S0140-6736(18)30472-0.
- [2] Zhang S. Sudden cardiac death in China: current status and future perspectives. *Europace*, Oct, 2015. DOI: 10.1093/europace/euv143.
- [3] Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A Scientific Statement from the international Liaison Committee on Resuscitation; the American Heart Association Emergency cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; the Council on Stroke. *Resuscitation*, Dec, 2008. DOI: 10.1016/j.resuscitation.2008.09.017.
- [4] Su CP, Wu JH, Yang MC, et al. Demographics and clinical features of postresuscitation comorbidities in long-term survivors of out-of-hospital cardiac arrest: A national follow-up study. *Biomed Res Int*, 2017. DOI: 10.1155/2017/9259182.
- [5] Tsai MS, Chiang WC, Lee CC, et al. Infections in the survivors of out-of-hospital cardiac arrest in the first 7 days. *Intensive Care Med*, May 31, 2005. DOI: 10.1007/s00134-005-2612-6.
- [6] Adrie C, Adib-Conquy M, Laurent I, et al. Successful cardiopulmonary resuscitation after cardiac arrest as a "sepsis-like" syndrome. *Circulation*, Jul 30, 2002. DOI: 10.1161/01.cir.0000023891.80661.ad.
- [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  
60



- 
- 1  
2  
3  
4 395 resuscitation may be beneficial for cardiac arrest. *Int J Cardiol*, Nov 1, 2016. DOI:  
5  
6 396 10.1016/j.ijcard.2016.08.017.  
7  
8  
9 397 [15] Niimura T, Zamami Y, Koyama T, et al. Hydrocortisone administration was  
10  
11 398 associated with improved survival in Japanese patients with cardiac arrest. *Sci Rep*,  
12  
13  
14 399 Dec 20, 2017. DOI: 10.1038/s41598-017-17686-3.  
15  
16  
17 400 [16] Chalkias A, Xanthos T. Post-cardiac arrest syndrome: mechanisms and evaluation  
18  
19 401 of adrenal insufficiency. *World J Crit Care Med*, Feb 4, 2012. DOI:  
20  
21 402 10.5492/wjccm.v1.i1.4.  
22  
23  
24 403 [17] Buddineni JP, Callaway C, Huang DT. Epinephrine, vasopressin and steroids for  
25  
26 404 in-hospital cardiac arrest: the right cocktail therapy? *Crit Care*, Jun 2, 2014. DOI:  
27  
28 405 10.1186/cc13903.  
29  
30  
31  
32 406 [18] Varvarousi G, Stefaniotou A, Varvaroussis D et al. Glucocorticoids as an emerging  
33  
34 407 pharmacologic agent for cardiopulmonary resuscitation. *Cardiovasc Drugs Ther*, Oct,  
35  
36 408 2014. DOI: 10.1007/s10557-014-6547-4.  
37  
38  
39  
40 409 [19] Kadmiel M, Cidlowski JA. Glucocorticoid receptor signaling in health and disease.  
41  
42 410 *Trends Pharmacol Sci*, Sep, 2013. DOI: 10.1016/j.tips.2013.07.003.  
43  
44  
45 411 [20] Norbiato G, Bevilacqua M, Vago T, et al. Cortisol resistance in acquired  
46  
47 412 immunodeficiency syndrome. *J Clin Endocrinol Metab*, Mar, 1992. DOI:  
48  
49 413 10.1210/jcem.74.3.1740494.  
50  
51  
52  
53 414 [21] Vassiliou AG, Floros G, Jahaj E, et al. Decreased glucocorticoid receptor  
54  
55 415 expression during critical illness. *Eur J Clin Invest*, Apr, 2019. DOI: 10.1111/eci.13073.  
56  
57  
58 416 [22] Alder MN, Opoka AM, Wong HR. The glucocorticoid receptor and cortisol levels  
59  
60

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

- 
- 1  
2  
3  
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  
22 468 [38] Vassiliou AG, Stamogiannos G, Jahaj E, et al. Longitudinal evaluation of  
23  
24 469 glucocorticoid receptor alpha/beta expression and signaling, adrenocortical function  
25  
26 470 and cytokines in critically ill steroid-free patients. *Mol Cell Endocrinol*, Feb 5, 2020.  
27  
28 471 DOI: 10.1016/j.mce.2019.110656.  
29  
30  
31  
32 472 [39] Indyk JA, Candido-Vitto C, Wolf IM, et al. Reduced glucocorticoid receptor  
33  
34 473 protein expression in children with critical illness. *Horm Res Paediatr. Horm Res*  
35  
36 474 *Paediatr*. 2013. DOI: 10.1159/000348290.  
37  
38  
39  
40 475 [40] Téblick A, Van Dyck L, Van Aerde N, et al. Impact of duration of critical illness  
41  
42 476 and level of systemic glucocorticoid availability on tissue-specific glucocorticoid  
43  
44 477 receptor expression and actions: A prospective, observational, cross-sectional human  
45  
46 478 and two translational mouse studies. *EBioMedicine*. 2022. DOI:  
47  
48 479 10.1016/j.ebiom.2022.104057  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

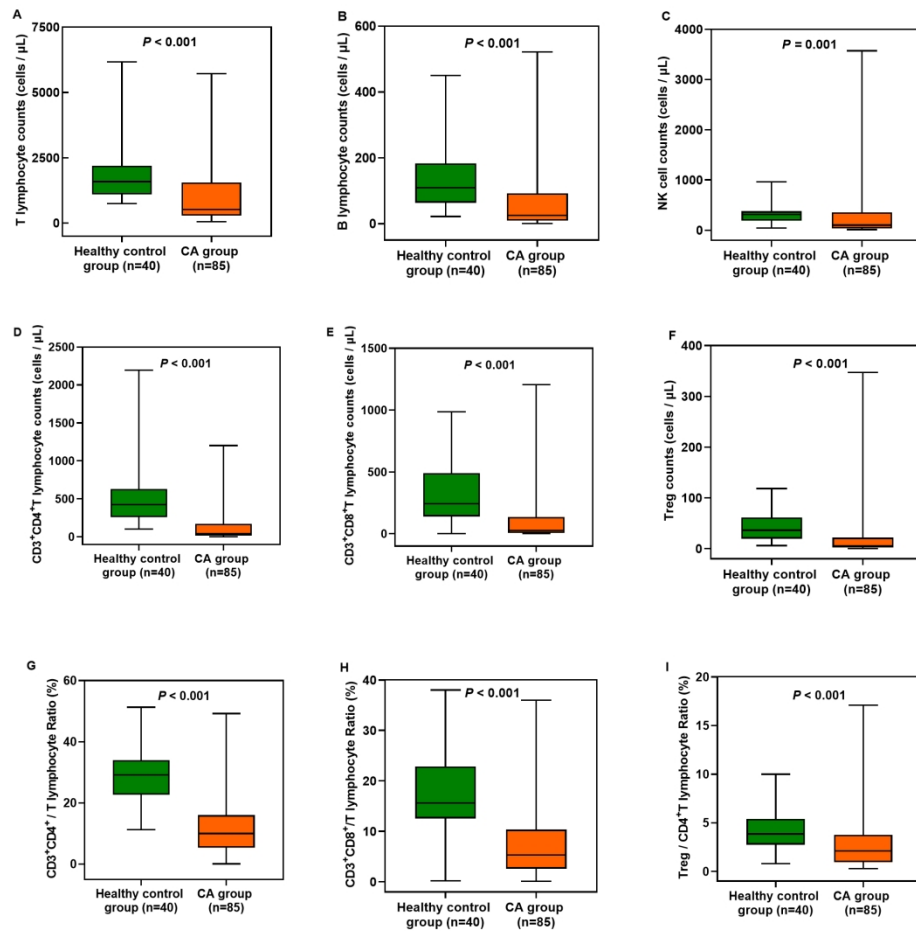
---

1  
2  
3  
4 480 **Figure legends**

5  
6 481 **Fig. 1.** Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts,  
7  
8  
9 482 CD3<sup>+</sup>CD4<sup>+</sup>/T, CD3<sup>+</sup>CD8<sup>+</sup>/T, and Treg/CD4<sup>+</sup>T lymphocyte ratios between the healthy  
10  
11 483 control group and CA group. The CA group showed significant differences compared  
12  
13  
14 484 with the healthy control group (P<0.001). CA, cardiac arrest; CD, cluster-of-  
15  
16  
17 485 differentiation; NK, natural killer; Treg, regulatory T.

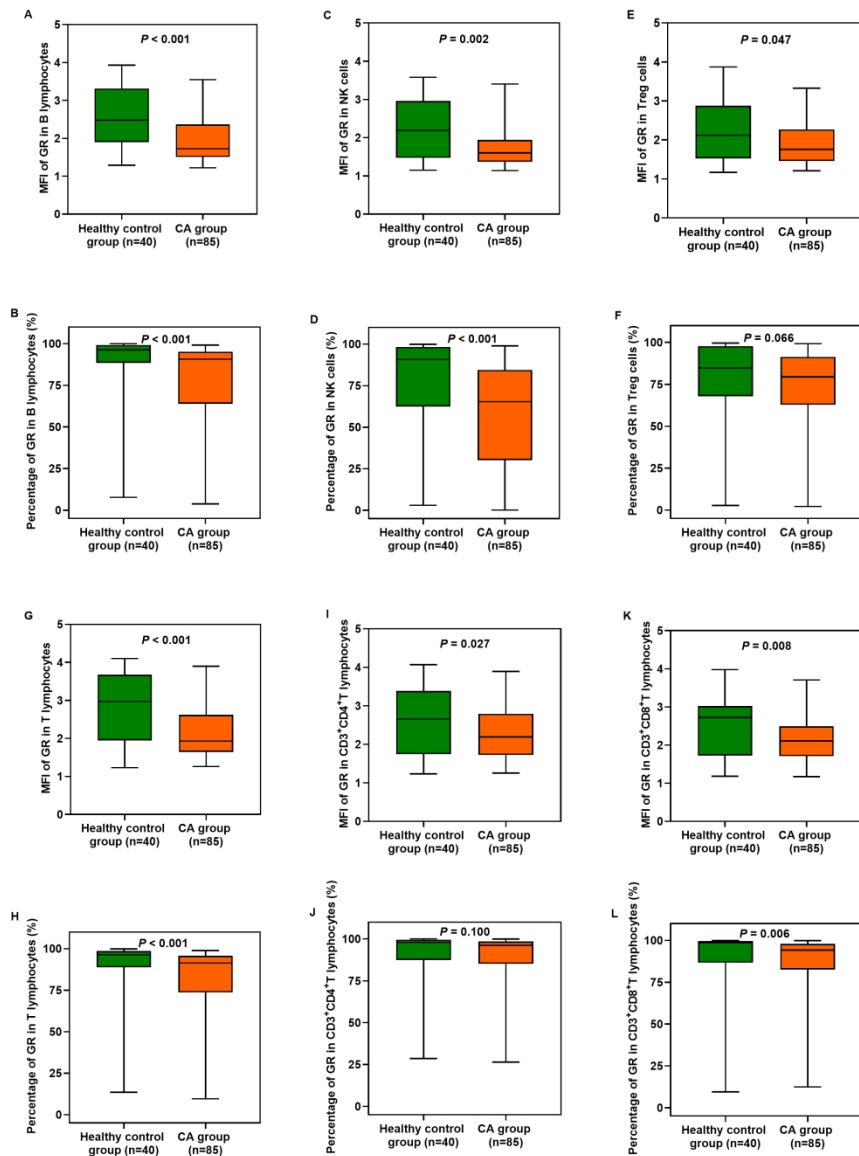
18  
19 486 **Fig. 2.** Expression of GRs in circulatory T and B lymphocytes, NK cells, and Treg cells  
20  
21  
22 487 in the healthy control group and CA group. The CA group showed significant  
23  
24  
25 488 differences compared with the healthy control group (P<0.05). CA, cardiac arrest; CD,  
26  
27  
28 489 cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; ROSC,  
29  
30 490 return of spontaneous circulation; Treg, regulatory T.

31  
32  
33 491 **Fig. 3.** (A, B) Plasma total cortisol and ACTH levels (the natural logarithmic  
34  
35  
36 492 conversion values after adding 1) after ROSC in the healthy control group and CA  
37  
38  
39 493 group. (C, D) Plasma total cortisol and ACTH levels in survivors and non-survivors  
40  
41  
42 494 after ROSC. The CA group showed significant differences compared with the healthy  
43  
44  
45 495 control group (P<0.05). ACTH, adrenocorticotrophic hormone; CA, cardiac arrest;  
46  
47  
48 496 ROSC, return of spontaneous circulation.



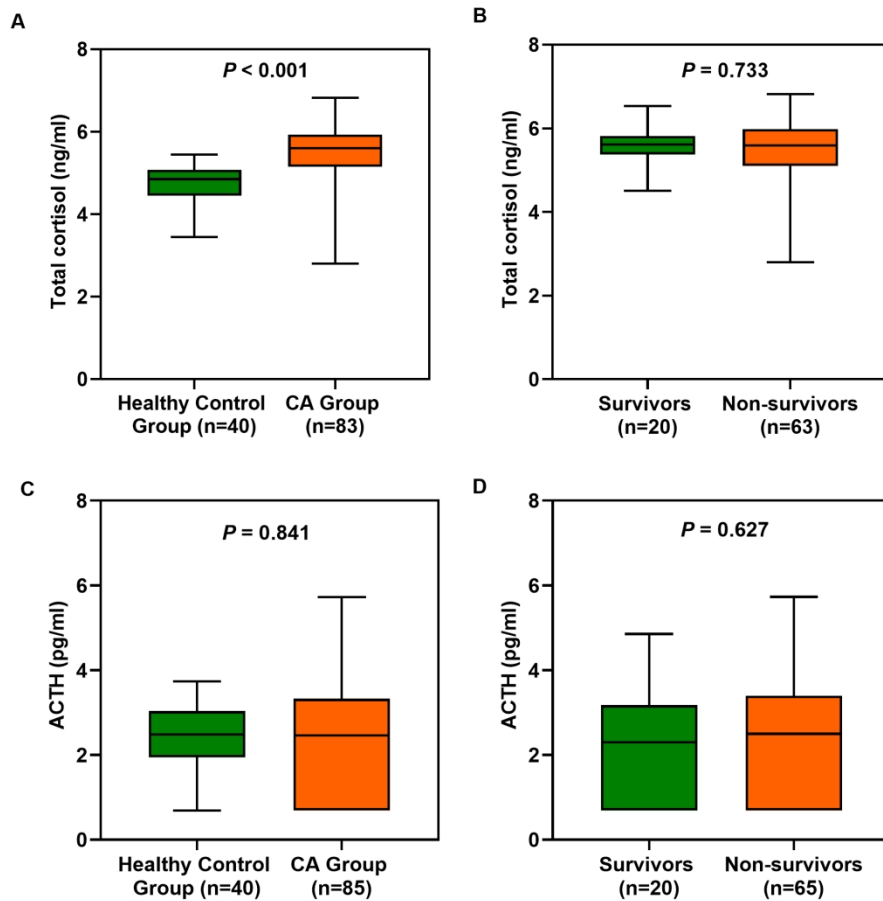
Changes in circulatory T and B lymphocyte, NK cell, and Treg cell counts, and CD3+CD4+/T, CD3+CD8+/T, and Treg/CD4+T lymphocyte ratios between the healthy control group and CA group. The CA group showed significant differences compared with the healthy control group ( $P < 0.001$ ). CA, cardiac arrest;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

Yanan Yu<sup>1</sup>; Ziren Tang<sup>1</sup>; Miaorong Xie<sup>2</sup>; Jiabao Li<sup>3</sup>; Chenchen Hang<sup>1</sup>; Le An<sup>1</sup>; Chunsheng Li<sup>1, \*</sup>

<sup>1</sup>Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

<sup>2</sup>Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing 100032, China

<sup>3</sup>Department of Critical Care, Beijing Friendship Hospital, Capital Medical University, Beijing 100020, China

\*Corresponding author: Chungsheng Li, M.D.

Department of Emergency Medicine, Beijing Chaoyang Hospital,

Capital Medical University, 8 Worker's Stadium South Road, Chaoyang District, Beijing 100020, China, Tel: +86

13681392380; E-mail: lcscy@163.com

**Contents**

Supplemental Figure 1

Supplemental Figure 2

Supplemental Table 1

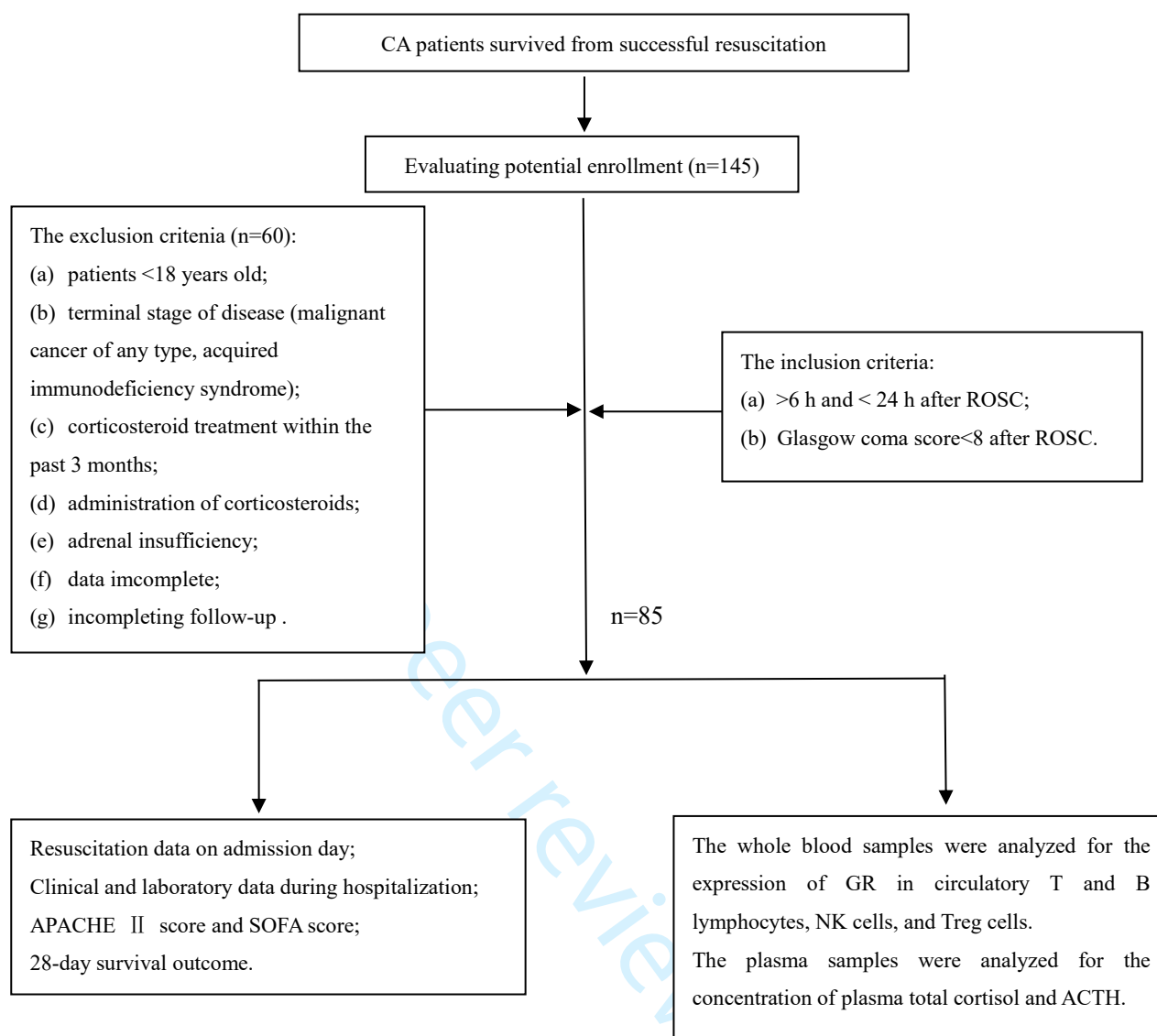
Supplemental Table 2

Supplemental Table 3

Supplemental Table 4

Supplemental Table 5

Supplemental Table 6



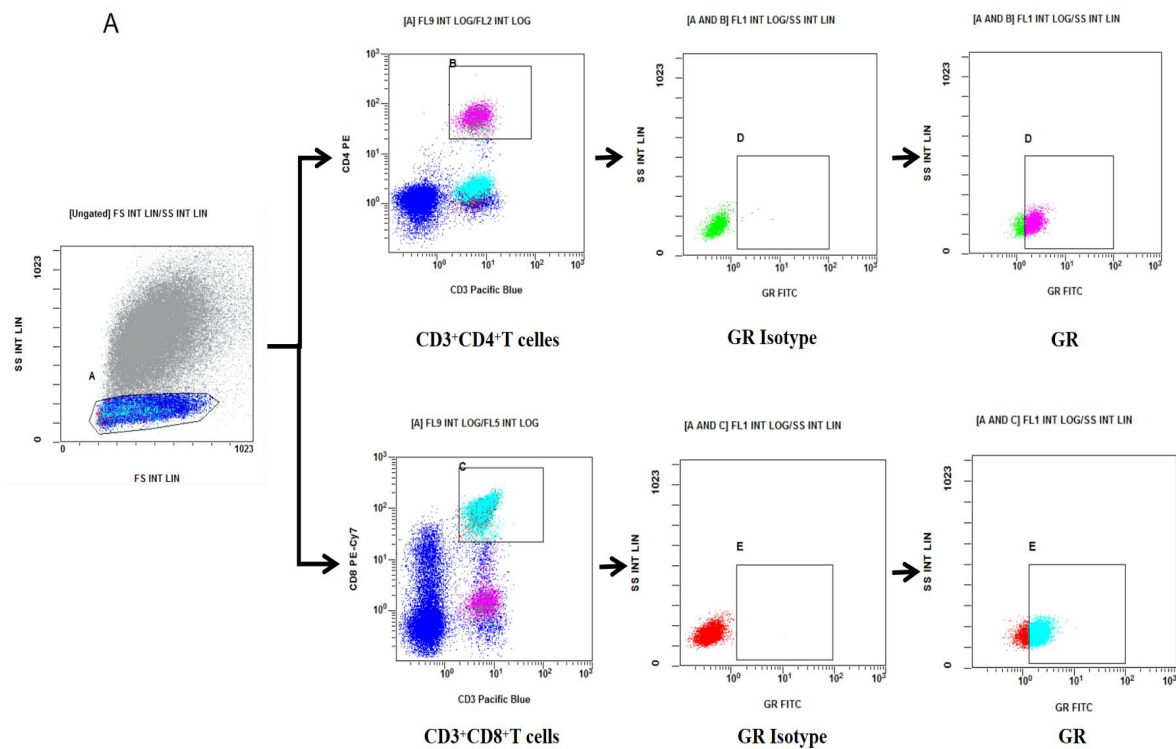
Supplemental Figure 1. The flow chart of the study.

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.

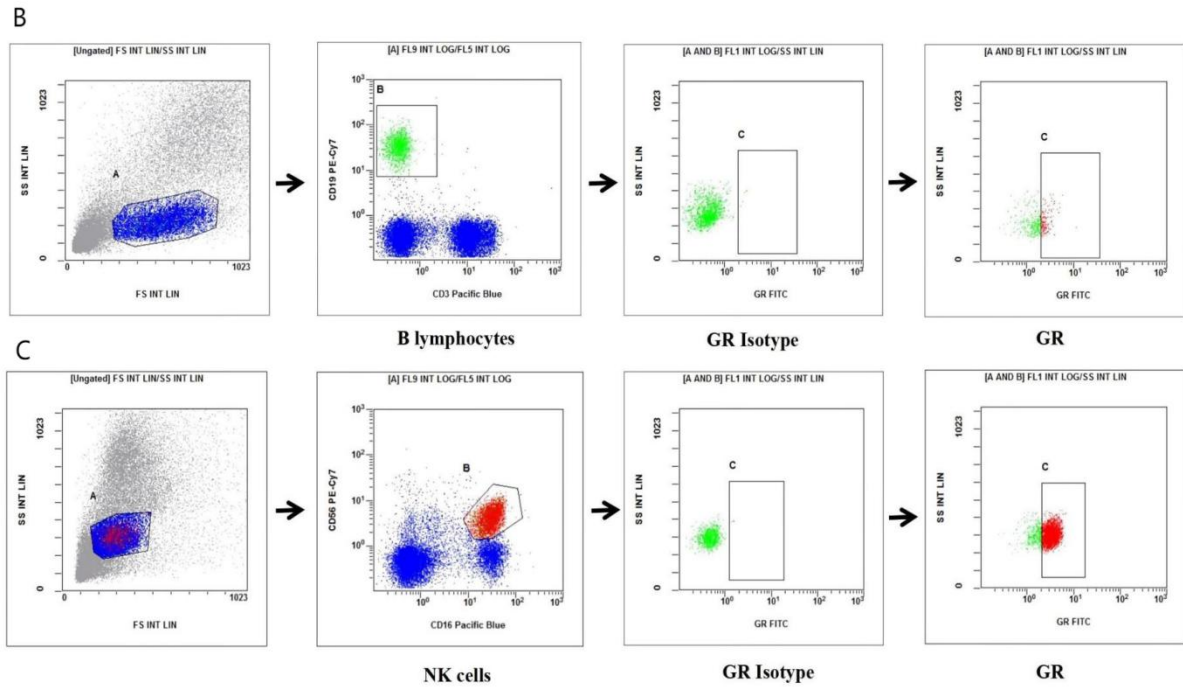
GR expression levels were determined on T cells, B cells, NK cells, and T regulatory (Treg) cells. Single cells were gated from all cellular events (FSC/SSC gate). B cells were identified as CD3<sup>-</sup>CD19<sup>+</sup> cells. NK cells were identified as CD16<sup>+</sup>56<sup>+</sup> cells. T cells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

#### A. Expression of GR on T cells

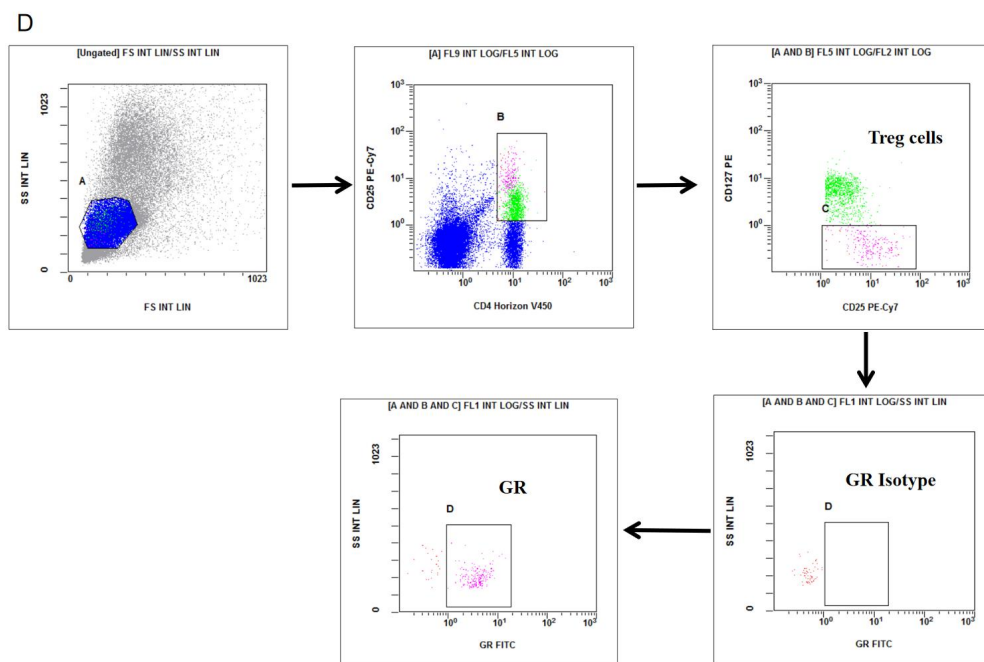


B. Expression of GR on B cells

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 <sup>a</sup>
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 <sup>b</sup>
Mouse IgG1 Isotype	MCA928F	FITC	Bio-Rad
Mouse IgG1, $\kappa$ Isotype	557872	PE-Cy7	BD Pharmingen
Mouse IgG1, $\kappa$ Isotype	554680	PE	BD Pharmingen
Mouse IgG1, $\kappa$ Isotype	558120	Pacific Blue	BD Pharmingen

<sup>a</sup> BD Pharmingen, San Diego, USA; <sup>b</sup> 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

	<b>Healthy Control Group (n=40)</b>	<b>Successful Resuscitation Group (n=85)</b>	<b>Z-value</b>	<b>P-value</b>
T lymphocyte count (cells / $\mu$ L)	1586.0 (1101.5, 2192.5)	514.0 (287.5, 1555.0)	-4.515	<0.001
NK cell count (/ $\mu$ L)	311.5 (191.0, 378.8)	101.0 (36.0, 351.5)	-3.332	0.001
B lymphocyte count (/ $\mu$ L)	109.3 (63.7, 183.3)	25.7 (9.4, 92.3)	-5.076	<0.001
Treg count (/ $\mu$ L)	0.259 (0.095, 0.516)	0.233 (0.135, 0.488)	-5.518	<0.001
Treg / CD4 <sup>+</sup> T lymphocyte Ratio	0.039 (0.028, 0.054)	0.021 (0.010, 0.038)	-4.418	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocyte count (/ $\mu$ L)	421.7 (258.6, 627.4)	38.9 (17.6, 168.3)	-6.256	<0.001
CD3 <sup>+</sup> CD4 <sup>+</sup> / T lymphocyte Ratio	0.292 (0.227, 0.340)	0.100 (0.054, 0.160)	-7.066	<0.001
CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocyte count (/ $\mu$ L)	241.1 (139.5, 488.6)	26.3 (7.2, 135.9)	-5.287	<0.001
CD3 <sup>+</sup> CD8 <sup>+</sup> / 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 <sup>+</sup> T lymphocyte Ratio	0.025 (0.009, 0.043)	0.021 (0.010, 0.034)	-0.498	0.619
CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocyte count (/μL)	55.1 (32.4, 228.0)	38.0 (16.0, 168.1)	-0.850	0.396
CD3 <sup>+</sup> CD4 <sup>+</sup> / T lymphocyte Ratio	0.118 (0.070, 0.236)	0.097 (0.049, 0.142)	-1.565	0.118
CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocyte count (/μL)	25.4 (12.5, 96.2)	26.3 (6.3, 138.8)	-0.021	0.983
CD3 <sup>+</sup> CD8 <sup>+</sup> / 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 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	0.980 (0.874, 0.996)	0.957 (0.824, 0.985)	-2.204	0.100
MFI of GR on CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	2.65 (1.75, 3.38)	2.17 (1.70, 2.92)	-1.646	0.027
Percentage of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocytes	0.986 (0.868, 0.996)	0.938 (0.823, 0.979)	-2.758	0.006
MFI of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> 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 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	0.975 (0.876, 0.985)	0.957 (0.845, 0.987)	-0.617	0.538
MFI of GR on CD3 <sup>+</sup> CD4 <sup>+</sup> T lymphocytes	2.08 (1.72, 3.35)	2.22 (1.71, 2.69)	-0.865	0.387
Percentage of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> T lymphocytes	0.963 (0.816, 0.977)	0.938 (0.834, 0.980)	-0.254	0.800
MFI of GR on CD3 <sup>+</sup> CD8 <sup>+</sup> 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
<b>Title and abstract</b>	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
<b>Introduction</b>			
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
<b>Methods</b>			
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	

Continued on next page

For peer review only

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
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
---------	----	---	----

\*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](http://www.strobe-statement.org).  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60