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[1]; Ziren Tang[1]; Miaorong Xie[2]; Jiabao Li[3]; Chenchen Hang[1]; Le An[1]; Chunsheng Li[1,*]

[1]Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

[2]Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing 100032, China

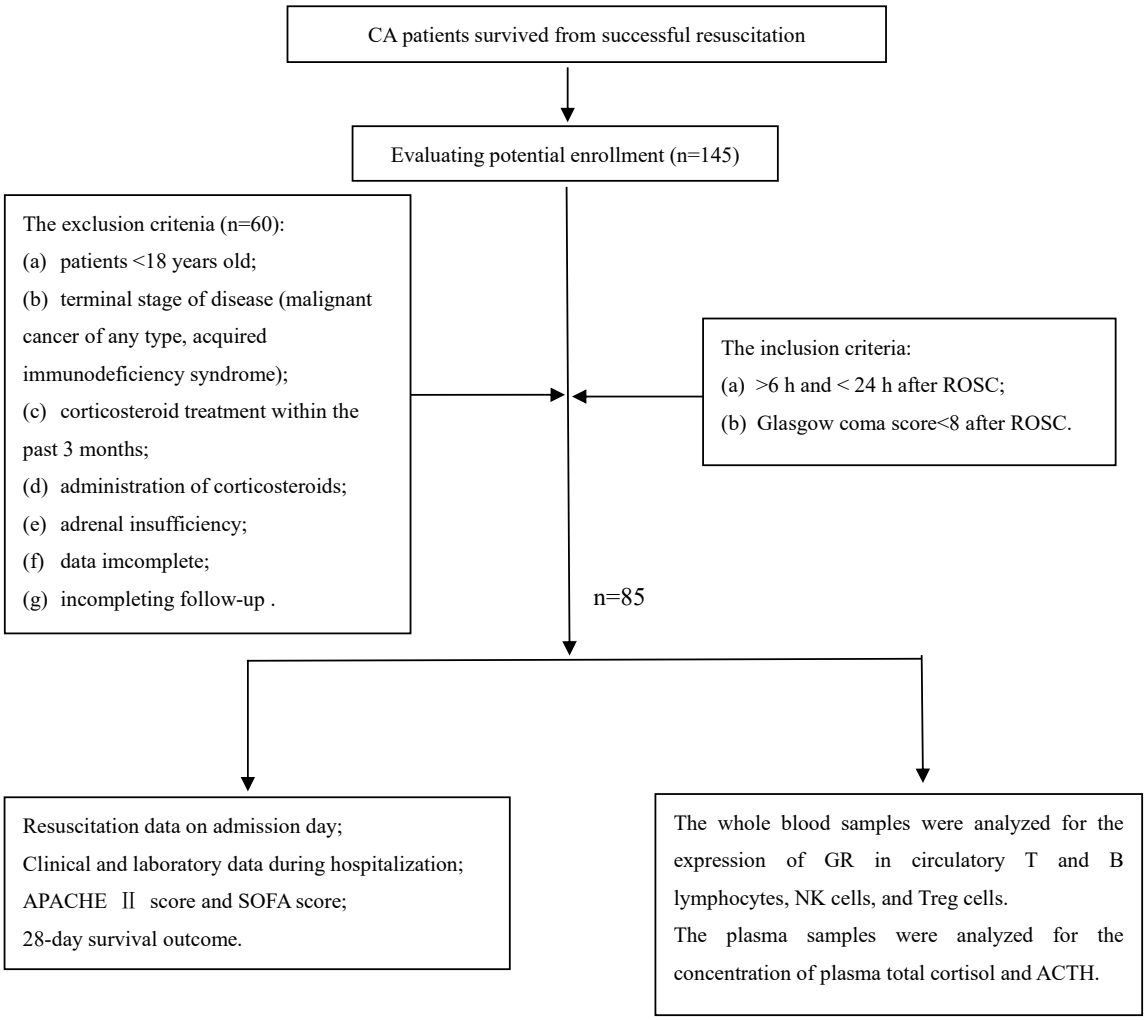
[3]Department of Critical Care, Beijing Friendship Hospital, Capital Medical University, Beijing 100020, China

*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: lcscyyy@163.com

Contents



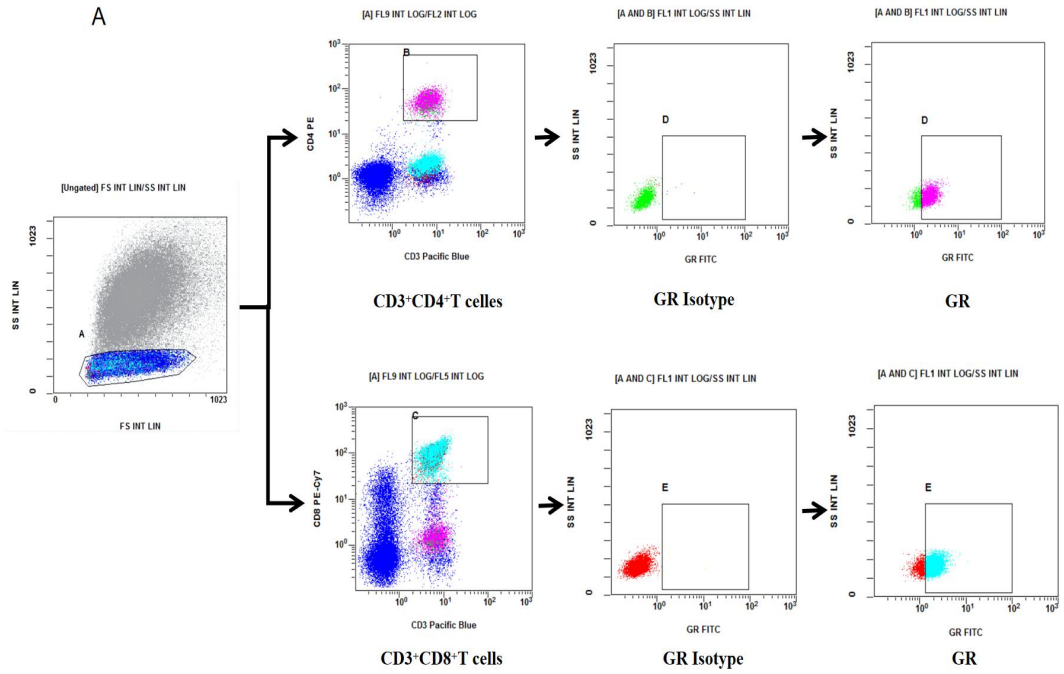
Supplemental Figure 1. The flow chart of the study.

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

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

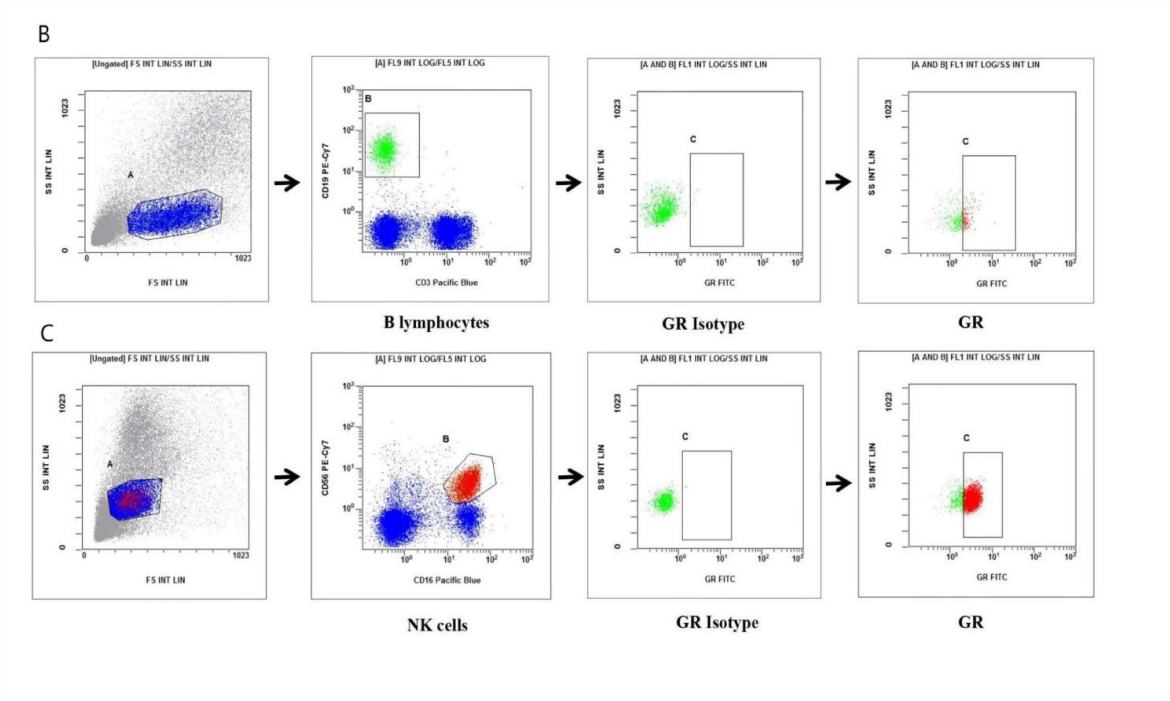
GR expression levels were determined on T cells, B cells, NK cells, and T regulatory (Treg) cells. Single cells were gated from all cellular events (FSC/SSC gate). B cells were identified as $CD3^{-}CD19^{+}$ cells. NK cells were identified as $CD16^{+}56^{+}$ cells. T cells were identified as $CD3^{+}CD4^{+}$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

A. Expression of GR on T cells

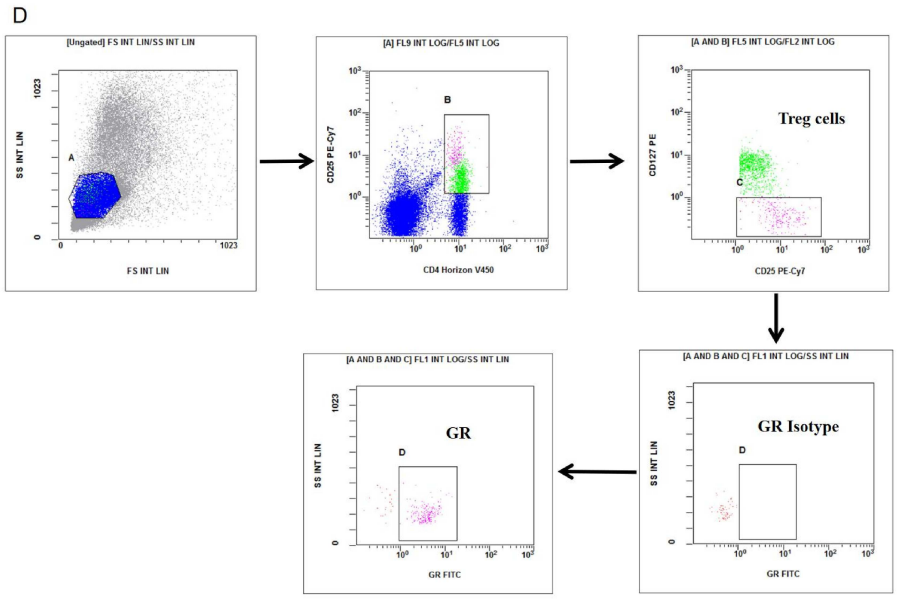


B. Expression of GR on B cells

C. Expression of GR on NK cells



D. Expression of GR on Treg cells



Supplemental Table 1. Details of antibodies for flow cytometry.

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

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

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

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

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

Data are presented as mean±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 /μL)	1586.0 (1101.5, 2192.5)	514.0 (287.5, 1555.0)	-4.515	<0.001
NK cell count (/μL)	311.5 (191.0, 378.8)	101.0 (36.0, 351.5)	-3.332	0.001
B lymphocyte count (/μL)	109.3 (63.7, 183.3)	25.7 (9.4, 92.3)	-5.076	<0.001
Treg count (/μL)	0.259 (0.095, 0.516)	0.233 (0.135, 0.488)	-5.518	<0.001
Treg / $CD4^+$T lymphocyte Ratio	0.039 (0.028, 0.054)	0.021 (0.010, 0.038)	-4.418	<0.001
$CD3^+CD4^+$ T lymphocyte count (/μL)	421.7 (258.6, 627.4)	38.9 (17.6, 168.3)	-6.256	<0.001
$CD3^+CD4^+$ / T lymphocyte Ratio	0.292 (0.227, 0.340)	0.100 (0.054, 0.160)	-7.066	<0.001
$CD3^+CD8^+$ T lymphocyte count (/μL)	241.1 (139.5, 488.6)	26.3 (7.2, 135.9)	-5.287	<0.001
$CD3^+CD8^+$ / T lymphocyte Ratio	0.157 (0.126, 0.229)	0.053 (0.026, 0.104)	-5.719	<0.001

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

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

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

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

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

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

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

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

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

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