**Electronic supplemental material**

**Expression of glucocorticoid receptors** **in the early period after the return of spontaneous circulation among patients who experienced cardiac arrest: A retrospective observational study**

Yanan Yu a, Ziren Tang a, Jiabao Li c, Miaorong Xie b, Chenchen Hang a, Le An a, Chunsheng Li a, \*

a Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, Beijing, China

b Department of Emergency Medicine, Beijing Friendship Hospital, Capital Medical University, Beijing, China

c Department of Critical Care, Beijing Friendship Hospital, Capital Medical University, Beijing 100020, China

\* **Corresponding author**: Chunsheng Li, PhD

Department of Emergency Medicine, Beijing Chaoyang Hospital, Capital Medical University, 8 Worker’s Stadium South Road, Chaoyang District, Beijing 100020, China. E-mail: lcscyyy@163.com

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CA patients survived from successful resuscitation

Evaluating potential enrollment

**The exclusion criteria:**

(a) patients <18 years old;

(b) terminal stage of disease (malignant cancer of any type, acquired immunodeficiency syndrome);

(c) corticosteroid treatment within the past 3 months;

(d) administration of corticosteroids;

(e) adrenal insufficiency.

**The inclusion criteria:**

1. ROSC 6 h;
2. Glasgow coma score<8 after ROSC

The whole blood samples were analyzed for the expression of GR in circulatory T and B lymphocytes, NK cells, and Treg cells.

The plasma samples were analyzed for the concentration of plasma total cortisol and ACTH.

Resuscitation data on admission day

Clinical and laboratory data during hospitalization

APACHE Ⅱ score and SOFA score

28-day survival outcome

Additional file 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.

Additional file 2. Details of antibodies

|  |  |  |  |
| --- | --- | --- | --- |
| **Antigen**  | **Catalog Number** | **Fluorescein Conjugate** | **Source** |
| CD3  | 558117 | Pacific Blue | BD Pharmingena |
| 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-Radb |
| Mouse IgG1 Isotype | MCA928F | FITC | Bio-Rad |
| Mouse IgG1,ĸ Isotype | 557872 | PE-Cy7 | BD Pharmingen |
| Mouse IgG1,ĸ Isotype  | 554680 | PE | BD Pharmingen |
| Mouse IgG1,ĸ 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.

Additional file 3. 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 Non-CardiacInitial resuscitation | 10 (50.0%)10 (50.0%) | 24 (36.9%)41 (63.1%) | 0.2970.297 |
| 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 (×109/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±SD | 27.8±6.6  | 34.4±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±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.

Additional file 4. The flow cytometry results of cell counts and ratios of healthy control group and successful resuscitation group

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Healthy Control** **Group** | **Successful Resuscitation Group** | **P** |
| T lymphocyte count (/μL)NK cell count (/μL)B lymphocyte count (/μL)Tregs count (/μL)Treg / CD4+T lymphocyte Ratio CD3+CD4+T lymphocyte count (/μL)CD3+CD4+ / CD3+T lymphocyte RatioCD3+CD8+T lymphocyte count (/μL)CD3+CD8+ / CD3+T lymphocyte Ratio | 1586.0 (1101.5, 2192.5)311.5 (191.0, 378.8)109.3 (63.7, 183.3)0.259 (0.095, 0.516)0.039 (0.028, 0.054)421.7 (258.6, 627.4)0.292 (0.227, 0.340)241.1 (139.5, 488.6)0.157 (0.126, 0.229) | 514.0 (287.5, 1555.0) 101.0 (36.0, 351.5) 25.7 (9.4, 92.3)0.233 (0.135, 0.488)0.021 (0.010, 0.038)38.9 (17.6, 168.3)0.100 (0.054, 0.160)26.3 (7.2, 135.9)0.053 (0.026, 0.104) | <0.001<0.001<0.001<0.001<0.001<0.001<0.001<0.001<0.001 |

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

Additional file 5. The flow cytometry results of cell counts and ratios of the CA patients on admission based on 28‑day survival

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Survivors**  | **Non-survivors** | **P** |
| T lymphocyte count (/μL)NK cell count (/μL)B lymphocyte count (/μL)Tregs count (/μL)Treg / CD4+T lymphocyte Ratio CD3+CD4+T lymphocyte count (/μL)CD3+CD4+ / T lymphocyte RatioCD3+CD8+T lymphocyte count (/μL)CD3+CD8+ / T lymphocyte Ratio | 502.0 (353.8, 1199.8)167.0 (29.8, 309.3)38.6 (15.7, 103.5)0.318 (0.145, 0.552)0.025 (0.009, 0.043)55.1 (32.4, 228.0)0.118 (0.070, 0.236)25.4 (12.5, 96.2)0.054 (0.033, 0.104) | 514.0 (282.5, 1891.0)100.0 (36.0, 404.0)19.2 (7.1, 65.7)0.212 (0.128, 0.479)0.021 (0.010, 0.034)38.0 (16.0, 168.1)0.097 (0.049, 0.142)26.3 (6.3, 138.8)0.053 (0.025, 0.104) | 0.852 0.828 0.5270.3960.6190.3960.1180.9830.852 |

All the data in Additional file 5 are represented as the median [IQR]; IQR: Interquartile Range; CD: cluster-of-differentiation; GR, glucocorticoid receptor; NK, natural killer; Treg, regulatory T.

Additional file 6. 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)** | P |
| Percentage of GR on B lymphocytesMFI of GR on B lymphocytesPercentage of GR on T lymphocytesMFI of GR on T lymphocytesPercentage of GR on NK cellsMFI of GR on NK cellsPercentage of GR on Treg cellsMFI of GR on Treg cellsPercentage of GR on CD3+CD4+T lymphocytesMFI of GR on CD3+CD4+T lymphocytesPercentage of GR on CD3+CD8+T lymphocytesMFI of GR on CD3+CD8+T lymphocytes | 0.963 (0.885, 0.992) 2.48 (1.91, 3.31) 0.964 (0.889, 0.986)2.98(1.95, 3.68)0.907 (0.624, 0.983)2.19 (1.48, 2.96)0.848 (0.680, 0.978)2.12 (1.53, 2.88)0.980 (0.874, 0.996)2.65 (1.75, 3.38)0.986 (0.868, 0.996)2.73 (1.73, 3.02) | 0.896 (0.605, 0.949) 1.73 (1.50, 2.37)0.900 (0.703, 0.955) 1.92 (1.36, 1.99)0.611 (0.306, 0.840)1.60 (1.36, 1.99)0.784 (0.589, 0.911)1.76 (1.44, 2.30)0.957 (0.824, 0.985)2.17 (1.70, 2.92)0.938 (0.823, 0.979)2.10 (1.68, 2.54) | <0.001<0.001<0.001<0.001<0.0010.002 0.0660.047 0.100 0.027 0.006 0.008  |

All the data in Additional file 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.

Additional file 7. The flow cytometry results of GR expression in the survivors and non-survivors.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Survivors** **(n=20)**  | **Non-survivors (n=65)** | **P** |
| Percentage of GR on B lymphocytesMFI of GR on B lymphocytesPercentage of GR on T lymphocytesMFI of GR on T lymphocytesPercentage of GR on NK cellsMFI of GR on NK cellsPercentage of GR on TregsMFI of GR on TregsPercentage of GR on CD3+CD4+T lymphocytesMFI of GR on CD3+CD4+T lymphocytesPercentage of GR on CD3+CD8+T lymphocytesMFI of GR on CD3+CD8+T lymphocytes | 0.904 (0.595, 0.976)1.92 (1.52, 2.54)0.899 (0.778, 0.969)2.05 (1.67, 2.83)0.717 (0.292, 0.886)1.54 (1.37, 2.09)0.780 (0.667, 0.849)1.61 (1.48, 2.30)0.975 (0.876, 0.985) 2.08 (1.72, 3.35)0.963 (0.816, 0.977)2.08 (1.68, 3.10) | 0.906 (0.657, 0.946)1.72 (1.51, 2.31)0.913 (0.692, 0.951)1.91 (1.64, 2.46)0.556 (0.302, 0.823)1.61 (1.34, 1.87)0.799 (0.576, 0.923) 1.77 (1.45, 2.27)0.957 (0.845, 0.987)2.22 (1.71, 2.69)0.938 (0.834, 0.980)2.11(1.71, 2.46) | 0.431 0.278 0.291 0.303 0.449 0.572 0.660 0.996 0.538 0.387 0.800 0.514  |

All the data in Additional file 7 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.