**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**

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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-Cardiac  Initial resuscitation | 10 (50.0%)  10 (50.0%) | 24 (36.9%)  41 (63.1%) | 0.297  0.297 |
| 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 Ratio  CD3+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 Ratio  CD3+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  0.527  0.396  0.619  0.396  0.118  0.983  0.852 |

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 lymphocytes  MFI of GR on B lymphocytes  Percentage of GR on T lymphocytes  MFI of GR on T lymphocytes  Percentage of GR on NK cells  MFI of GR on NK cells  Percentage of GR on Treg cells  MFI of GR on Treg cells  Percentage of GR on CD3+CD4+T lymphocytes  MFI of GR on CD3+CD4+T lymphocytes  Percentage of GR on CD3+CD8+T lymphocytes  MFI 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  <0.001  0.002  0.066  0.047  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 lymphocytes  MFI of GR on B lymphocytes  Percentage of GR on T lymphocytes  MFI of GR on T lymphocytes  Percentage of GR on NK cells  MFI of GR on NK cells  Percentage of GR on Tregs  MFI of GR on Tregs  Percentage of GR on CD3+CD4+T lymphocytes  MFI of GR on CD3+CD4+T lymphocytes  Percentage of GR on CD3+CD8+T lymphocytes  MFI 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.