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Predicting outcome in patients under extracorporeal membrane oxygenation due to cardiogenic shock through dynamic change of lymphocytes and interleukins

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ABSTRACT

Objectives: Peripheral blood parameters implicating the irreversible damages of cardiogenic shock under extracorporeal membrane oxygenation (ECMO) support patients were investigated.
Methods: The blood was collected from 23 cardiogenic shock cases at the time of ECMO installation, and 2, 6, 12, and 24 h after oxygenation. Plasma levels of IL-2, IL-6, IL-7, IL-8, IL-10, IFN-γ, MCP-1, and carbonic anhydrase IX (CA IX) were determined by ELISA. Reactive oxygen species were measured by luminol and lucigenin and leukocyte subpopulations were analyzed by flow cytometry. Generalized additive models (GAMs) were performed to identify the death ranges of every variable and the variables were further discretized at each time point. The combination of predictors was selected from both original and discretized covariates by the generalized linear model (GLM) at each time point.
Results: Plasma IL-10 level was the most distinct biomarker between survivors and non-survivors after oxygenation. IL-10 sequentially partnered with IL-6, IL-7, and lymphocyte percentage at 2 h, with $CD3^+CD4^+$ T cells at 6 h, with CA IX at 12 h, and $CD3^+$ T lymphocytes at 24 h predicted death with AUCs 1.000, 0.975, 0.992, and 0.992, respectively.
Conclusions: Combination of the GAM plots and GLM models overcame the complexity of different disease categories. The systemic irreversible damages from cardiogenic shock ECMO cases might be detected from several peripheral blood parameters.

1. Introduction

Cardiogenic shock (CS) is typically the result of severe left ventricular contractile failure which causes systemic anoxia, hypoxia, and ischemia. The extreme stress has been proposed to stimulate sympathetic nerve to release catecholamine in acute myocardial infarction (AMI) patients with 2.5- to 3-fold elevation than the controls [1], and catecholamine has been shown to directly stimulate the promoter/enhancer of IL-10 by targeting a cAMP responsive element (CRE) in monocytes, but not in T-cells, after AMI without preceding inflammation [2,3]. Rapid monocytic IL-10 release after sympathetic activation may represent a common pathway for immunosuppression induced by stress. The extracorporeal membrane oxygenation (ECMO) support has been used for CS rescue. IL-10 and superoxide ions were shown to predict death/survival of ECMO therapy for CS patients as early as at installation before resuscitation [4]. Our hypothesis is that the effects of CS and ECMO therapy are systemic; therefore, peripheral blood dynamic changes might shed light of body response, particular the different response between death and survival. Dynamic changes of reactive oxygen species (ROS), peripheral blood leukocyte subpopulations, plasma cytokines and carbonic anhydrase IX, a hypoxia marker [5,6], were investigated in CS ECMO cases within 24 h after ECMO treatment and the differences between survivors and non-survivors were also analyzed in this study.

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2. Materials and methods

2.1. Study population

This study was approved by the Research Ethics Committee (REC) of the National Taiwan University Hospital. ECMO was indicated for patients with circulatory collapse or needing inotrope support of more than 40 inotrope equivalents (=dopamine + dobutamine + epinephrine × 100 + norepinephrine × 100, μg/kg/min) [7]. The ECMO system (Medtronic Inc.) was usually set up through the femoral veno-arterial route [7]. Inclusion criteria included age ≥18 year and cardiogenic shock that needed ECMO support. Exclusion criteria included pre-existing multiple organ failure, severe brain insult before ECMO, and the absence of signed consent for study enrollment. The blood was separately collected at ECMO installation before resuscitation and 2, 6, 12, and 24 h after oxygenation for the study.

Patients who survived for more than 7 days after the initial insult and ECMO treatment were defined as survival, and death patients were defined as mortality or multiple organ failure incompatible with life within 7 days after ECMO was installed. To focus on the effects of CS rescued by ECMO, the survival time was defined as more than 7 days to avoid the complications after the rescue. Irreversible damages from CS often cause multiple organ failure; therefore, multiple organ failure incompatible with life within 7 days after ECMO was defined as death, failure of rescue.

2.2. ROS determined by luminol and lucigenin

The blood was collected into a sodium heparin Vacutainer tube, was kept on ice and was analyzed within 1 h. Total ROS activity was measured by 0.2 mL of blood reacting with 1 mL of 0.3 mM luminol for 4 min at 37 °C in a Chemiluminescence Analyzer (Tohoku Electronic Industrial). The plasma superoxide ion measurement was done by reacting 0.2 mL of blood with 1 mL of 0.1 mM lucigenin at 37 °C for 4 min.

2.3. IL-2, IL-6, IL-7, IL-8, IL-10, interferon-γ (IFN-γ), monocyte chemotactic protein-1 (MCP-1), and CA IX

The plasma concentrations of IL-2, IL-6, IL-8, IL-10, interferon-γ (IFN-γ), and MCP-1 were separately analyzed using commercial ELISA kits (Becton Dickinson, BD). The IL-7 ELISA kits were from BioLegend. Plasma CA IX levels were also measured by a commercial ELISA kit (R&D Systems).

2.4 . Flow cytometric analysis

The blood was collected into a Vacutainer tube containing EDTA. One hundred microliters of blood per tube was separately stained with four or three or two-color combinations of CD19 APC/CD3 PerCP/CD4 FITC/CD8 PE, CD3 PerCP/CD16 FITC/CD56 PE, CD14 PE/CD16 FITC (Becton Dickinson) at room temperature for 25 min in the dark, lysed with BD lysing solution, washed two times with phosphate buffer saline (PBS) containing 1% heat-inactivated fetal bovine serum, and fixed with PBS containing 0.25% paraformaldehyde. Cells were analyzed and 20,000 events were collected per assay by a BD Calibur flow cytometer with CellQuest software version 3.2.

2.5. Statistical analysis

R software (http://www.r-project.org/), version 2.15.2, was used for the analysis of the generalized additive models (GAMs) for simple logistic regression and the generalized linear model (GLM) for maximum-likelihood estimation. Due to wide distributions of IL-2, IL-6, IL-7, IL-8, IL-10, IFN-γ, MCP-1, CA IX, ROS determined by luminol and by lucigenin, the log values of these variables were used for GAM and GLM analysis. The GAM plot was generated after smoothing each component. The X-axis represents the covariate, and the Y-axis is logit (probability of death) or equivalently, the log value of the odds; Y >0 for death, Y <0 for survival. Case distributions were shown as short vertical lines along the X-axis. The prediction power was conducted by GLM analysis. The β is the estimated regression coefficient; se is the estimated standard error of β; VIF is the variance inflating factor; R^2 is the adjusted generalized R^2 (Nagelkerke R^2); the modified Hosmer–Lemeshow goodness-of-fit test, a statistical test for goodness of fit for logistic regression model, was abbreviated as the H-L GOF test. The positive value of β indicated that the higher the value the worse condition the patient could be. The variance inflation factor (VIF) is a simple diagnostic of collinearity, one for each regression coefficient (other than the intercept). Values of VIF exceeding 5 were considered evidence of collinearity. The p-value was further calculated by the likelihood ratio (LR) test. The $p < 0.05$ was considered significant.

3. Results

3.1. Demographic and clinical characteristics of patients

Total 23 patients, including 11 AMI, 6 dilated cardiomyopathy (DCMP), 4 acute myocarditis (AM), 1 arrhythmia, and 1 aortic dissection cases, were enrolled from January 2010 to November 2011. The demographic and clinical characteristics are presented in Table 1. The detail information was described before [4].

3.2. Death ranges were discretized for every variable according to its generalized additive model plot at each time point

To overcome the complexity of AMI, DCMP, and AM disease categories, the generalized additive model (GAM) was chosen. Computing with GAM by R was separately conducted for every variable at 0, 2, 6, 12, and 24 h; resultant death ranges were discretized accordingly. The analysis and prediction results at 0 h were reported before [4].

3.3. Outcome prediction at 2 h: IL-10, IL-6, IL-7, and lymphocytes with AUC 1.000

Once the ECMO support was initiated, reperfusion started. The response to I/R was systemic; whole blood ROS separately determined by luminol and lucigenin, plasma IL-2, IL-6, IL-7, IL-8, IL-10, IFN-γ, MCP-1, and CA IX levels determined by ELISA, lymphocytes, monocytes, and granulocytes in percentages of white blood cells (WBC), as well as $CD19^+$ B, $CD3^+$ T, $CD3^+CD4^+$ T, $CD3^+CD8^+$ T, $CD16^+CD56^+$ natural killer (NK), and $CD56^+$ NK T cells presented in both percentages of WBC and lymphocytes by flow cytometry, were separately performed and

Table 1
Demographic and clinical characteristics of cardiogenic extracorporeal membrane oxygenation (ECMO) patients.

Variable	Survival	Death	p-Value
Sample size (n)	12 (52.2%)	11 (47.8%)	
Age (years)	44.96 ± 12.95	55.68 ± 19.63	0.166
Gender			>0.999
Male	9 (50.0%)	9 (50.0%)	
Female	3 (60.0%)	2 (40.0%)	
WBC (K/μL)	13.28 ± 6.46	11.47 ± 4.96	0.347
Diagnosis group			0.856
AMI (n = 11)	5 (45.5%)	6 (54.5%)	
DCMP (n = 6)	3 (50.0%)	3 (50.0%)	
Myocarditis (n = 4)	3 (75.0%)	1 (25.0%)	
Others (n = 2)	1 (50.0%)	1 (50.0%)	

Notes: The sample statistics presented in this table were mean ± standard deviation (SD) for continuous variables and frequency (percentage, %) for categorical variables. The listed p-values of statistical tests were calculated using the Wilcoxon rank-sum test for continuous variables and the Fisher's exact test for categorical variables.