The Value of Extracellular Cold-inducible RNA-binding Protein (eCIRP) in Predicting the Severity and Prognosis of Patients After Cardiac Arrest

Ling Wang
Department of Emergency Medicine,First Affiliated Hospital of Dalian Medical University
Rui-Fang Li
Department of Emergency Medicine,First Affiliated Hospital of Dalian Medical University
Xiao-Lan Guan
Department of Emergency Medicine,First Affiliated Hospital of Dalian Medical University
Shuang-Shuang Liang
Department of Emergency Medicine,First Affiliated Hospital of Dalian Medical University
Ping Gong (gongp828@sina.cn)
Department of Emergency Medicine, First Affiliated Hospital of Dalian Medical University, Dalian City, Liaoning Province, China https://orcid.org/0000-0001-9910-7948

Research

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Abstract

Background: Extracellular cold-inducible RNA-binding protein (eCIRP) acting as a novel damage-associated molecular pattern molecule promotes systemic inflammatory responses, including neuroinflammation in cerebral ischemia. We aimed to observe the changes of serum eCIRP and evaluate whether the increased serum eCIRP was associated with the severity and prognosis in patients with restoration of spontaneous circulation (ROSC).

Methods: A total of 73 patients after ROSC were divided into non-survivor (*n* = 48) and survivor (*n* = 25) groups based on 28-day survival. Healthy volunteers (*n* = 25) were enrolled as controls. Serum eCIRP, procalcitonin (PCT), the pro-inflammatory mediators tumor necrosis factor (TNF)-α, interleukin-6 (IL)-6 and high mobility group protein (HMGB1), the neurological damage biomarkers neuron-specific enolase (NSE) and soluble protein 100β (S100β) were measured on days 1, 3 and 7 after ROSC. Clinical data and laboratory findings were collected, and the Sequential Organ Failure Assessment (SOFA) score and Acute Physiology and Chronic Health Evaluation (APACHE II) were calculated concurrently. Cerebral performance category (CPC) scores on day 28 after ROSC were recorded.

Results: Serum eCIRP, PCT, NSE and S100β, IL-6, TNF-α and HMGB1 were significantly increased within the first week after ROSC. The increased levels of eCIRP was positively correlated with IL-6, TNF-α, HMGB1, NSE, S100β, lactate, CPR time, SOFA score and APACHE II score after ROSC. Serum eCIRP on days 1, 3 and 7 after ROSC could predict 28-day mortality and neurological prognosis. Serum eCIRP on day 3 after ROSC had a biggest AUC [0.862 (95%CI: 0.741-0.941)] for 28-day mortality and a biggest AUC [0.807 (95%CI: 0.630-0.981)] for neurological prognosis.

Conclusions: Systemic inflammatory response with increased serum eCIRP occurred in patients after ROSC. Increased eCIRP level was positively correlated with the aggravation of systemic inflammatory response and the severity after ROSC. Serum eCIRP serves as a potential predictor for 28-day mortality and poor neurological prognosis after ROSC.

Introduction

The annual incidence of sudden cardiac arrest (CA) in adults is about 550,000 in the United States [1]. The prognosis of CA patients after restoration of spontaneous circulation (ROSC) remains dismal despite of public training of cardiopulmonary resuscitation (CPR) and recent improvement in therapeutic approaches [2, 3]. Survival in patients with out-of-hospital cardiac arrest (OHCA) is <15% [2], whereas survival with in-hospital cardiac arrest (IHCA) is approximately 22% [3]. For patients survived from initial resuscitation, the mortality is still high in the post-resuscitation phase due to a complex pathophysiological process so-called post–cardiac arrest syndrome (PACS), which includes post-cardiac arrest brain injury, post–cardiac arrest myocardial dysfunction, systemic ischemia/reperfusion response, and persistent precipitating pathology [4]. The systematic inflammatory response after ROSC, which is induced by systemic ischemia/reperfusion and characterized by the absence of endotoxin in plasma,

high levels of circulating cytokines and inflammatory mediators, mimics the process in sepsis [5]. This "sepsis-like" inflammatory response has been demonstrated to contribute to vital organs injury such as brain and heart [5, 6], which is closely associated with neurological disability and high mortality. So far, there is limited evidence to accurately predict the PACS severity and prognosis in patients after ROSC.

Cold-inducible RNA binding protein (CIRP) belongs to the family of cold shock proteins which are constitutively but weakly expressed in various tissues [7, 8]. Intracellular CIRP (iCIRP) plays a vital role in a variety of cellular stress responses, including mRNA stability, cell proliferation, cell survival, circadian clock gene modulation, telomerase maintenance, stress adaptation, and tumor formation and progression [7]. During hypoxia and inflammation, CIRP is translocated from the nucleus to the cytoplasm and gradually released to the extracellular space, which is called extracellular CIRP (eCIRP) [9]. Recent evidence showed that eCIRP is a proinflammatory cytokine that can induce various inflammatory reactions, including neuroinflammation in cerebral ischemia [9-12]. Unlike other neurological biomarkers such as neuron-specific enolase (NSE) and soluble protein 100β (S100β), the role of eCIRP in hypoxic–ischemic encephalopathy has not been fully discovered [12, 13]. It has been demonstrated that eCIRP binds to the toll like receptor (TLR) 4-myeloid differentiation protein (MD) 2/nuclear factor-κB (NF-κB) leading to the induction and release of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin-6 (IL)-6 and high mobility group protein (HMGB1) [10, 14-16], which is involved in systematic inflammatory response.

The role of eCIRP in PACS for patients after ROSC is not well understood. However, increased serum levels of eCIRP in patients with hemorrhagic shock and sepsis correlated with organ injury and poor prognosis [10, 11]. Similarly, serum and tissue levels of eCIRP were also increased in a number of organ-targeted ischemia and reperfusion models characterized by sterile inflammation, including rodent models of hepatic ischemia, mesenteric ischemia, ischemic acute kidney injury, and stroke [12, 16-18]. We hypothesized that eCIRP levels in PACS may provide some clues for the clinical outcomes. This study aimed to determine the dynamic changes of serum eCIRP and other proinflammatory mediators, and to clarify the predictive value of serum eCIRP for the severity and prognosis in patients after ROSC.

Methods

Enrolled participants and grouping

This prospective study was conducted in the emergency intensive care unit (ICU) and cardiac ICU in the First Affiliated Hospital of Dalian Medical University (Dalian, China). Patients successfully resuscitated from CA from January 1, 2017 to December 30, 2019, were enrolled. All enrolled patients received treatments upon ICU admission according to the 2015 International Consensus on Cardiopulmonary Resuscitation [19]. Enrolled patients were subdivided into two groups (survivors and non-survivors) on the basis of 28-day survival. Concurrently, sex- and age-matched healthy adult volunteers were enrolled as a control group. The present study was carried on in accordance with the Declaration of Helsinki (2013 edition) adopted by the World Medical Association [20]. The study was approved by the Medical Ethics

Committee of the First Affiliated Hospital of Dalian Medical University (PJ-KS-KY-2019-151) and written informed consent was obtained from their legal guardians.

Inclusion and exclusion criteria

Adult patients (over 18 years old) resuscitated from OHCA and IHCA were included. Patients were excluded if they had severe craniocerebral trauma or acute cerebrovascular disease, systemic inflammatory or autoimmune disorders upon hospital admission, past histories of corticosteroids medication and other systemic diseases such as hematological diseases, uremia, liver cirrhosis and malignancies, and were pregnant or in the period of lactation.

Data collection

Clinical data were prospectively collected, including demographic data, medical history, the length of ICU stay, causes of CA, initial heart rhythm, CPR time, laboratory findings and outcomes. The Sequential Organ Failure Assessment (SOFA) score and Acute Physiology and Chronic Health Evaluation (APACHE II) were calculated on days 1, 3 and 7 after ROSC on the basis of age, medical history, vital signs and laboratory results. Cerebral performance category (CPC) scores on day 28 after ROSC were recorded to assess neurological prognosis. CPC of 1-2 was defined as a good neurological outcome while CPC of 3-5 was defined as a poor outcome.

Measurement of biomarkers

Venous blood was sampled from the patients on days 1, 3 and 7 after ROSC or when enrolled for healthy volunteers. Blood samples were centrifuged at 1000 *g* at 4°C for 15 min; and then the isolated serum was stored at −80°C for further analyses. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure serum eCIRP (CUSABIO, Wuhan, China), NSE (CUSABIO, Wuhan, China), S100β (CUSABIO, Wuhan, China), IL-6 (Elabscience, Wuhan, China), TNF-α (Elabscience, Wuhan, China) and HMGB1 (Elabscience, Wuhan, China) in accordance with the manufactures' instructions.

Statistical analyses

Data were analyzed and graphed using SPSS v22.0 (IBM, Armonk, NY, USA) and GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA). The results were reported as counts (percentage) for the categorical variables, mean ± SD if normally distributed and median (interquartile range) if not normally distributed for the continuous variables. Pearson chi-squared or Fisher exact tests were used (as appropriate) to compare demographic variables. Repeated-measure analysis of variance (ANOVA) was used to compare the changes of variables at different time points among the survivors, non-survivors and healthy volunteers, followed by Bonferroni test for multiple comparisons. Bivariate correlation analyses between eCIRP and other variables were conducted using Spearman's correlation coefficient. For determining associations between serum eCIRP and outcome, we generated receiver operating characteristic (ROC) curves and the areas under the ROC curves (AUCs) were calculated; AUCs were statistically compared using DeLong's test. On the basis of the optimal thresholds determined by

analyzing ROC curves, prognostic parameters (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], Youden Index, positive likelihood ratio [LR+] and negative likelihood ratio [LR−]) were also calculated. Differences were considered statistically significant when $P<0.05$.

Results

Baseline characteristics

A total of 73 patients after ROSC and 25 healthy volunteers were enrolled. There were no significant differences in age and gender among healthy volunteers, survivors and non-survivors (all $P > 0.05$, Table 1). The causes of CA and previous medical history in non-survivors ($n = 48$) and survivors ($n = 25$) were not significantly different. However, the non-survivors had less ventricular arrhythmia, more asystole and pulseless activity as initial rhythm and shorter duration of ICU-stay when compared to the survivors (all $P < 0.05$). The CPR time, SOFA score and APACHE II score in the non-survivors were significantly higher than those in the survivors (all $P < 0.05$). A total of 60% survivors had a 28-day CPC of 1-2 scores, indicating a good neurological prognosis.

Levels of serum procalcitonin (PCT), brain natriuretic peptide, lactate, creatinine, WBC count and neutrophil proportion at baseline were significantly higher in patients undergoing CPR when compared with those in healthy volunteers (all $P < 0.05$), whereas baseline levels of serum WBC count and neutrophil proportion were similar in the survivors and non-survivors. Additionally, serum PCT, brain natriuretic peptide, lactate and creatinine in non-survivors were significantly higher than those in the survivors (all $P < 0.05$).

Comparison of serum levels of eCIRP, IL-6, TNF-α, HMGB1 and PCT among healthy volunteers, survivors and non-survivors

Serum levels of eCIRP, IL-6, TNF-α and PCT were increased significantly after ROSC when compared with those in healthy volunteers (all $P < 0.05$, Figure 1). Moreover, serum levels of eCIRP, IL-6 and PCT in non-survivors on days 1, 3 and 7 after ROSC were significantly higher than those in survivors (all $P < 0.05$). Serum levels of TNF-α in the non-survivors on days 1 and 3 after ROSC were significantly higher than that in the survivors (both $P < 0.05$), but there were no significant differences on day 7 after ROSC. Serum levels of HMGB1 on day 1 after ROSC were similar to healthy volunteers, whereas serum levels of HMGB1 on days 3 and 7 after ROSC were significantly increased (both $P < 0.05$). In addition, serum levels of HMGB1 in the non-survivors on days 3 and 7 after ROSC were significantly higher than those in survivors (both $P < 0.05$).

Comparison of serum levels of NSE and S100β among healthy volunteers, survivors and non-survivors

Serum levels of NSE and S100β were increased significantly after ROSC when compared with healthy volunteers (both $P < 0.05$, Figure 2). Moreover, serum levels of NSE and S100β in non-survivors on days 1, 3 and 7 after ROSC were significantly higher than those in survivors (all $P < 0.05$).

Correlation of serum eCIRP with serum IL-6, TNF-α, HMGB1, NSE, S100β, lactate, CPR time, SOFA score and APACHE II score

Serum eCIRP was positively correlated with serum IL-6, TNF-α, NSE, S100β, lactate, CPR time, SOFA score and APACHE II score on days 1, 3 and 7 after ROSC and with serum HMGB1 on days 3 and 7 after ROSC (all $P < 0.05$, Table 2).

The predictive value of serum eCIRP for 28-day mortality and 28-day neurological prognosis

ROC curves of serum eCIRP on days 1, 3 and 7 after ROSC for predicting 28-day mortality and 28-day neurological prognosis were displayed in Figure 3. Serum levels of eCIRP on days 1, 3 and 7 after ROSC had a predictive value for 28-day mortality. Particularly, serum levels of eCIRP on day 3 after ROSC had a biggest AUC [0.862 (95%CI: 0.741-0.941)] (all $P < 0.05$, Figure 3A). Similarly, serum levels of eCIRP on days 1, 3 and 7 after ROSC had a predictive value for 28-day neurological prognosis, but serum levels of eCIRP on day 3 after ROSC had a biggest AUC [0.807 (95%CI: 0.630-0.981)] (all $P < 0.05$, Figure 3B).

Table 3 showed the cut-off value, sensitivity, specificity, PPV, NPV, Youden Index, LR+, and LR− for serum eCIRP on days 1, 3 and 7 after ROSC.

Discussion

Systematic sterile inflammatory response is a typical manifestation following ROSC [4]. The present study found that serum eCIRP were significantly increased in patients after ROSC especially in the non-survivors. The increased eCIRP was closely correlated with the changes of pro-inflammatory mediators and neurological biomarkers. More importantly, early stage eCIRP had a good predictive performance for the 28-day mortality and neurological outcome in patients with ROSC.

Levels of pro-inflammatory mediators including IL-6, TNF-α and HMGB1 well reflect post-cardiac arrest immuno-inflammatory response [21, 22]. Previous studies found that blood IL-6 level was significantly increased in CA patients surviving >24 h and this increase was closely associated with the severity of PCAS, organ dysfunction and mortality after ROSC [23, 24]. Our findings validated that serum IL-6, TNF-α, and HMGB1 were significantly increased after ROSC, with higher levels in non-survivors than survivors. It is of note that serum PCT was previously reported to be associated with PCAS, which is consistent with our finding that non-survivors bear higher serum PCT concentrations than survivors [25].

CIRP as a stress response protein migrates from the nucleus to the cytoplasm under hypoxia and inflammation after CA and subsequently becomes eCIRP [26]. eCIRP as a damage-associated molecular pattern molecule (DAMP) promotes inflammatory responses [9, 10]. TLR4 as one of the most widely studied pattern recognition receptors has been demonstrated to recognize and combine with eCIRP and subsequently mediate inflammatory responses [27]. In the current study, we observed that serum eCIRP was significantly increased in patients after ROSC and were higher in non-survivors than in survivors. We speculate that eCIRP promoting inflammatory response after ROSC may be related to NF-κB activation

through TLR4 signal pathway. This assumption was supported by the fact that serum eCIRP was positively correlated with inflammatory cytokines IL-6, TNF-α and HMGB1 within the first week after ROSC. Likewise, previous studies on hemorrhagic shock and sepsis revealed that eCIRP stimulated the release of TNF-a, IL-6 and HMGB1 from macrophages via TLR4/MD2 complex binding and NF-kB activation [9, 10, 14]. In addition, in animal models of sepsis and hemorrhagic shock, application of anti-CIRP antibody restrained CIRP-induced release of TNF-α and HMGB1, attenuated organ injury and ultimately prolonged survival [15, 28].

The severity of PCAS after ROSC is largely dependent on the duration of global ischemia, subsequent systemic inflammatory response syndrome (SIRS) and organ impairment [5, 29]. The APACHE II and SOFA scores representing the severity of PCAS and organ dysfunction were significantly higher in non-survivors than survivors after ROSC in this study, which was consistent with previous findings [30, 31]. The serum eCIRP was positively correlated with these two scoring systems and the potential prognostic value of eCIRP was also demonstrated in sepsis [11].

The multiple organ dysfunction was parallel with the changes of serum cytokines and inflammatory mediators in post-CA period [4]. Organ injury biomarkers including the serum brain natriuretic peptide, creatinine, NSE and S100β were significantly elevated after ROSC especially in the non-survivors. This phenomenon indicated a systemic ischemia following ROSC in vital organs (eg., heart, brain and kidney). Likewise, serum eCIRP has been shown to be positively linked with the aggravation of systemic inflammatory response and the severity of patients in rheumatoid arthritis and osteoarthritis [32, 33]. Hence, it is not surprising that serum eCIRP on day 1, 3 and 7 after ROSC could predict 28-day mortality, with value on day 3 had a biggest AUC. These findings suggested that serum eCIRP could serve as a screening tool for the early prediction of mortality in patients after ROSC.

Brain injury is well accepted as a major cause of post-resuscitative mortality and poor neurological outcomes after CA [34]. NSE and S100β are the most commonly used biomarkers of brain injury after CA [35]. In the present study, we observed that serum NSE and S100β in non-survivors more significantly rose in the non-survivors after ROSC, which was consistent with previous findings [36]. Both serum NSE and S100β were also positively correlated with serum eCIRP within the first week after ROSC. Serum eCIRP serves as an pro-inflammatory mediator that fuels neuroinflammation through inducing TNF-α and IL-1β production observed in microglia under hypoxic/ischemic stress [12, 13], which was in contrast to iCIRP that protected neural cell from subsequent oxidative stresses [37, 38]. eCIRP-induced neuroinflammation involved in resuscitation-induced blood-brain barrier (BBB) disruption, thus contributing to the prognosis of patients after ROSC [39]. We here proved that serum levels of eCIRP at the early stage after ROSC predicted 28-day neurological prognosis.

There are several limitations in the present study. First, eCIRP was assessed via peripheral blood samples, which may not completely reflect its status in organs such as brain and heart. Second, the specificity of eCIRP as a pro-inflammatory mediator in assessing the severity and predicting prognosis of patients after CA is limited due to the complex pathways and mediators involved in the PCAS. Third, a combination of

clinical manifestations and biomarkers may provide a more ideal predictive value for prognosis in the early phase of treatment [40]. Fourth, the expression of CIRP is affected under mild hypothermia condition [41], whereas we did not include patients receiving mild hypothermia and the correlation between mild hypothermia and eCIRP was not investigated. Fifth, the sample size was relatively small because of the short study period and strict exclusion criteria. Further large-scale studies are warranted to confirm the prognostic value of eCIRP in patients survived from CA.

Conclusions

Increased circulatory eCIRP is accompanied with a systemic inflammatory response in patients after ROSC. Elevated blood eCIRP was positively correlated with the aggravation of systemic inflammatory response and the severity of the patients after ROSC. In addition, blood eCIRP can serve as a potential predictor for 28-day mortality and poor neurological prognosis after ROSC.

Abbreviations

APACHE: Acute Physiology and Chronic Health Evaluation; AUC: area under the ROC curve; CA: cardiac arrest; CPC: cerebral performance category; CPR: cardiopulmonary resuscitation; eCIRP: extracellular cold-inducible RNA-binding protein; HMGB1: high mobility group protein; ICU: intensive care unit; IHCA: in-hospital cardiac arrest; IL: interleukin; LR+: positive likelihood ratio; LR−: negative likelihood ratio; MD: myeloid differentiation protein; NF-κB: nuclear factor-κB; NPV: negative predictive value; NSE: neuron-specific enolase; OHCA: out-of-hospital cardiac arrest; PACS: post–cardiac arrest syndrome; PCT: procalcitonin; PPV: positive predictive value; ROC: receiver operating characteristic; ROSC: restoration of spontaneous circulation; S100β: soluble protein 100β; SOFA: Sequential Organ Failure Assessment; TLR: toll like receptor; TNF: tumor necrosis factor.

Declarations

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Authors' contributions

PG and LW conceived and designed the experiments. LW, R-FL, X-LG and S-SL carried out the experiments. LW and R-FL analyzed the data. LW wrote the manuscript. PG took overall responsibility for the manuscript. All authors approved the final version of the manuscript.

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Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Medical Ethics Committee of the First Affiliated Hospital of Dalian Medical University (Dalian, China). Written informed consent was obtained from all patients (or their legal guardians) upon their initial admission to hospital and from healthy volunteers.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Baseline characteristics of enrolled participants on admission

	Healthy volunteers	Survivors	Non-survivors
	(*n* = 25)	(*n* = 25)	(*n* = 48)
Age (years)	58.9 ± 13.1	59.2 ± 16.3	63.9 ± 15.8
Male [*n* (%)]	13 (52.0%)	19 (76.0%)	29 (60.4%)
Previous medical history [*n* (%)]			
Diabetes	—	6 (24.0%)	12 (25.0%)
Hypertension	—	10 (40.0%)	23 (47.9%)
Coronary heart disease	—	11 (44.0%)	19 (39.6%)
Cerebrovascular disease	—	4 (16.0%)	6 (12.5%)
Chronic pulmonary disease	—	3 (12.0%)	4 (8.3%)
Chronic kidney disease	—	3 (12.0%)	5 (10.4%)
Post-operation	—	2 (8.0%)	3 (6.3%)
Cardiac arrest cause [*n* (%)]			
Cardiac	—	13 (52.0%)	22 (45.8%)
Respiratory	—	4 (16.0%)	11 (22.9%)
Cerebral	—	3 (12.0%)	8 (16.7%)
Others	—	5 (20.0%)	7 (14.6%)
Initial cardiac rhythm [*n* (%)]			
Ventricular arrhythmia	—	14 (56.0%)	4 (8.3%)[a]
Asystole and pulseless activity	—	11 (44.0%)	44 (91.7%)[a]
CPR time (min)	—	6.0 (2.5-10.0)	14.0 (7.0-23.0)[a]
Length of ICU stay (days)	—	9 (6-17)	4 (1-11)[a]
Laboratory findings			
WBC ($\times 10^9$/L)	6.84 (5.71-8.59)	12.46 (8.48-18.60)[b]	14.10 (9.63-22.38)
Neutrophil ratio (%)	57.9 (50.6-66.8)	84.0 (75.9-90.8)[b]	85.9 (78.8-91.3
PCT (ng/mL)	0.18 (0.01-0.36)	1.36 (0.69-1.94)[b]	2.01 (1.05-3.37)[a]
Lactate (mmol/L)	0.59 (0.31-1.32)	2.00 (1.35-4.60)[b]	6.10 (2.43-11.23)[a]
Creatinine (μmol/L)	68.2 (56.3-79.5)	83.0 (60.0-104.5)[b]	142.5 (100.3-234.3)[a]

BNP (pg/mL)	30.8 (22.3-45.9)	119.7 (48.5-345.9)[b]	615.8 (243.6-1885.3)[a]
APACHE II score	—	20 (13.5-38.0)	38.0 (34.3-41.0)[a]
SOFA score	—	3.5 (4.0-7.5)	10.0 (8.3-13.0)[a]
28-day CPC (1-2) [*n* (%)]	—	15 (60.0%)	—

Note: Values are the mean ± standard deviation or median (range). APACHE II, Acute Physiology and Chronic Health Evaluation II; BNP, brain natriuretic peptide; CPC, cerebral performance category; CPR, cardiopulmonary resuscitation; ICU, intensive care unit; PCT, procalcitonin; SOFA, Sequential Organ Failure Assessment; WBC, white blood cell.

[a] $P < 0.05$ *vs* survivors, [b] $P < 0.05$ *vs* healthy volunteers.

Table 2 Correlation of serum eCIRP with serum IL-6, TNF-α, HMGB1, Lactate, NSE, S100β, CPR time, SOFA score and APACHE II score

Variables	Serum eCIRP	
	r	*P*
Day 1		
IL-6	0.380	0.001
TNF-α	0.325	0.005
HMGB1	0.085	0.476
Lactate	0.309	0.008
NSE	0.329	0.004
S100β	0.335	0.004
CPR time	0.303	0.009
SOFA score	0.438	0.000
APACHE II score	0.427	0.000
Day 3		
IL-6	0.341	0.012
TNF-α	0.343	0.011
HMGB1	0.386	0.004
Lactate	0.407	0.002
NSE	0.376	0.005
S100β	0.402	0.003
CPR time	0.273	0.046
SOFA score	0.495	0.000
APACHE II score	0.485	0.000
Day 7		
IL-6	0.341	0.038
TNF-α	0.308	0.042
HMGB1	0.455	0.002
Lactate	0.422	0.004
NSE	0.441	0.003
S100β	0.352	0.019

CPR time	0.330	0.029
SOFA score	0.459	0.002
APACHE II score	0.520	0.000

Note: APACHE II, Acute Physiology and Chronic Health Evaluation II; CPR**,** cardiopulmonary resuscitation; eCIRP, extracellular cold-inducible RNA-binding protein; HMGB1, high mobility group protein 1; IL-6, interleukin-6; NSE, neuron-specific enolase; S100β, soluble protein 100β; SOFA, Sequential Organ Failure Assessment; TNF-α, tumor necrosis factor-α.

Table 3A Performance of serum eCIRP on days 1, 3 and 7 after ROSC for predicting 28-day mortality

	Cut-off	Sensitivity	Specificity	PPV	NPV	Youden	LR+	LR−
	(pg/mL)	(%)	(%)	(%)	(%)	(%)		
eCIRP$_{D1}$	291.7	66.7	76.0	84.2	54.3	42.8	2.78	0.44
eCIRP$_{D3}$	463.6	93.1	84.0	87.1	91.3	77.1	5.82	0.08
eCIRP$_{D7}$	431.3	84.2	88.0	84.2	88.0	72.2	7.02	0.18

Note: eCIRP$_{D1}$, extracellular cold-inducible RNA-binding protein on day 1 after ROSC; eCIRP$_{D3}$, extracellular cold-inducible RNA-binding protein on day 3 after ROSC; eCIRP$_{D7}$, extracellular cold-inducible RNA-binding protein on day 7 after ROSC; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; ROSC, restoration of spontaneous circulation.

Table 3B Performance of serum eCIRP on days 1, 3 and 7 after ROSC for predicting 28-day neurological prognosis

	Cut-off	Sensitivity	Specificity	PPV	NPV	Youden	LR+	LR−
	(pg/mL)	(%)	(%)	(%)	(%)	(%)		
eCIRP$_{D1}$	190.1	80.0	66.7	61.5	83.3	46.7	2.40	0.30
eCIRP$_{D3}$	378.9	60.0	86.7	75.0	76.5	46.7	4.50	0.46
eCIRP$_{D7}$	315.7	70.0	93.3	87.5	82.4	63.3	10.5	0.32

Note: eCIRP$_{D1}$, extracellular cold-inducible RNA-binding protein on day 1 after ROSC; eCIRP$_{D3}$, extracellular cold-inducible RNA-binding protein on day 3 after ROSC; eCIRP$_{D7}$, extracellular cold-inducible RNA-binding

protein on day 7 after ROSC; LR+, positive likelihood ratio; LR−, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; ROSC, restoration of spontaneous circulation.

Figures

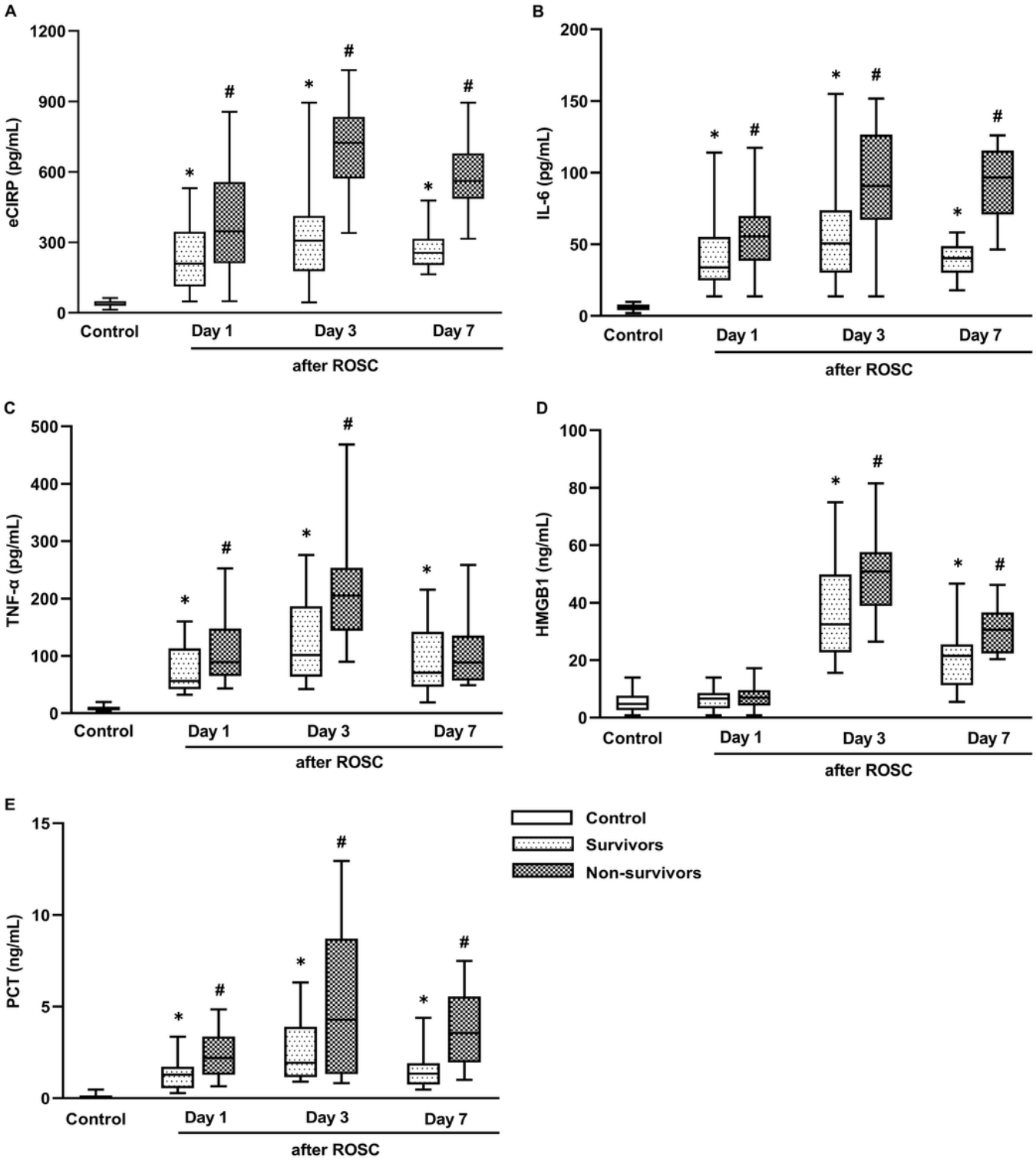

Figure 1

Comparison of serum levels of eCIRP, IL-6, TNF-α, HMGB1 and PCT in healthy volunteers (control), survivors and non-survivors. eCIRP, extracellular cold-inducible RNA-binding protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; HMGB1, high mobility group protein 1; PCT, procalcitonin; ROSC, restoration of spontaneous circulation. *P < 0.05 vs healthy volunteers; #P < 0.05 vs survivors.

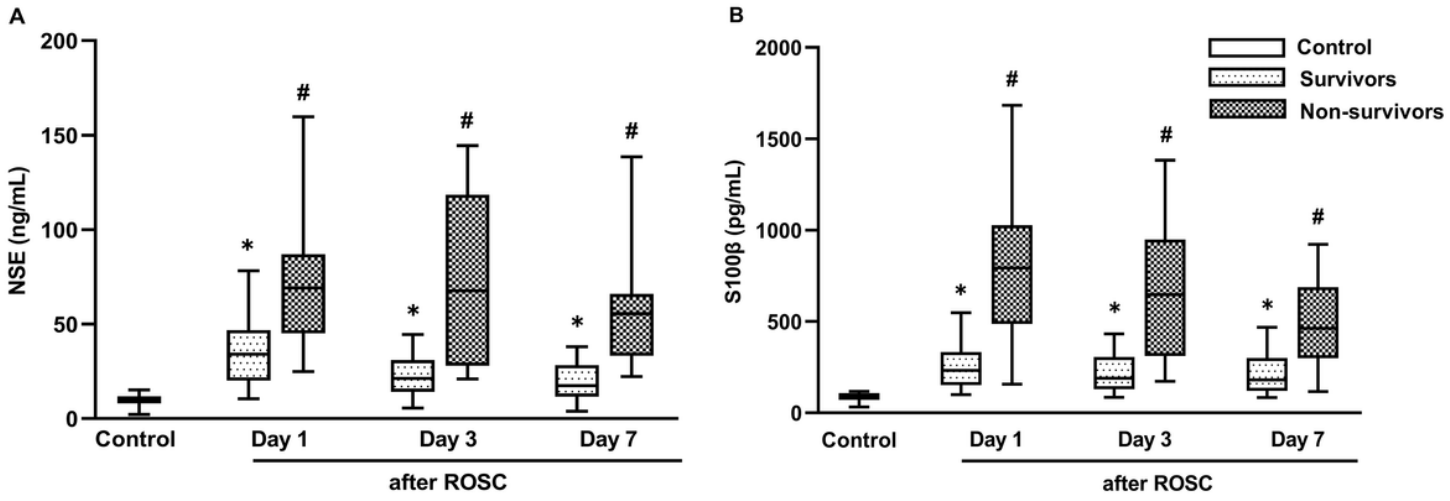

Figure 2

Comparison of serum levels of NSE and S100β in healthy volunteers (control), survivors and non-survivors. NSE, neuron-specific enolase; S100β, soluble protein-100β; ROSC, restoration of spontaneous circulation. *P < 0.05 vs healthy volunteers; #P < 0.05 vs survivors.

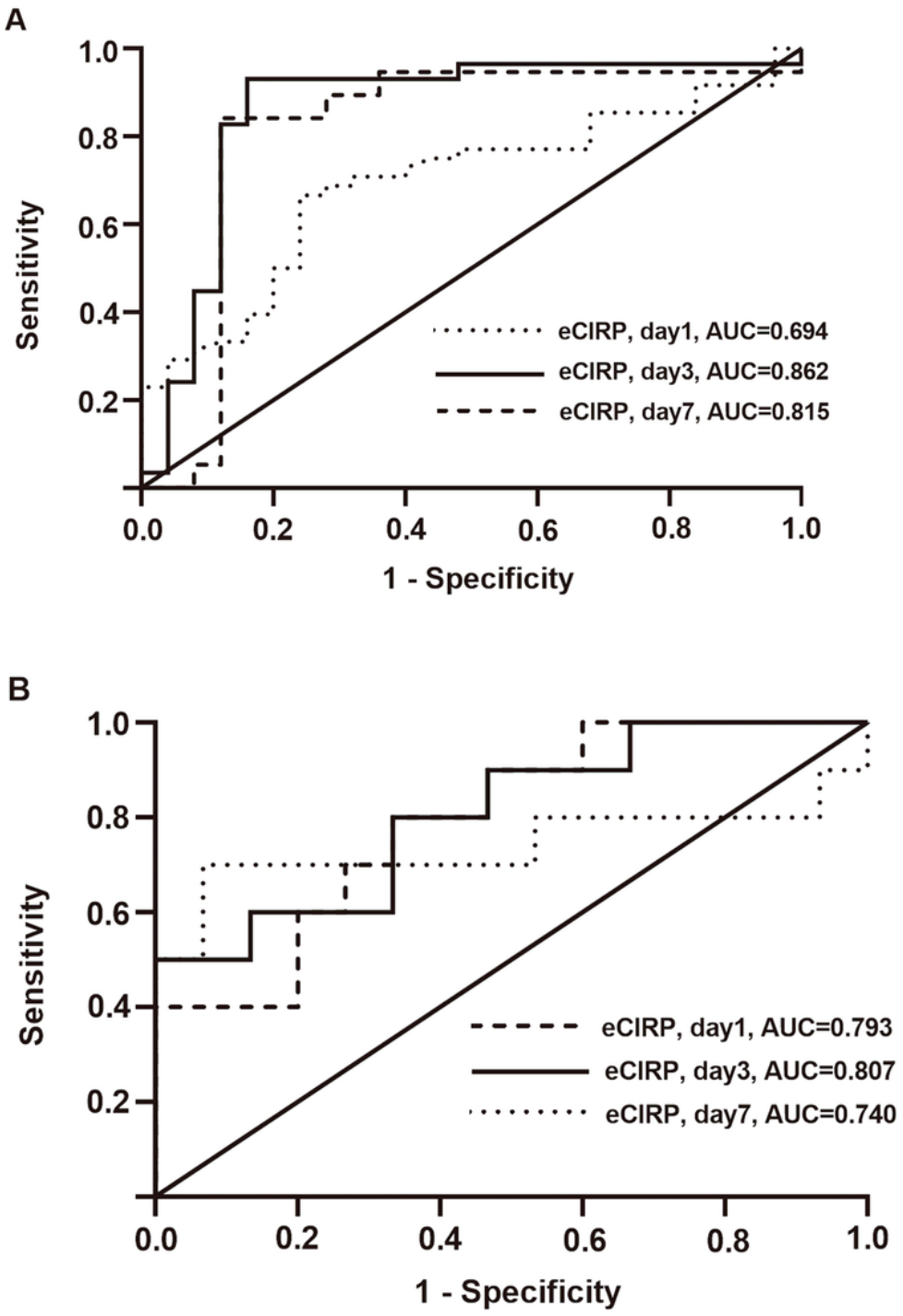

Figure 3

Receiver operating characteristic curves of serum eCIRP on days 1, 3 and 7 after ROSC for predicting 28-day mortality (A) and 28-day neurological prognosis (B). AUC, areas under the curves; eCIRP, extracellular cold-inducible RNA-binding protein.