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# Early predictive value of lipocalin-type prostaglandin D synthase for 28-day mortality in cardiac arrest patients: study protocol for a prospective study

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## ABSTRACT

**Introduction** Public training in cardiopulmonary resuscitation and treatment in emergency and intensive care unit have made tremendous progress. However, cardiac arrest remains a major health burden worldwide, with brain damage being a significant contributor to disability and mortality. Lipocalin-type prostaglandin D synthase (L-PGDS), which is mainly localised in the central nervous system, has been previously shown to inhibit postischemia neuronal apoptosis. Therefore, we aim to observe whether serum L-PGDS can serve as a potential biomarker and explore its role in determining the severity and prognosis of patients who have achieved restoration of spontaneous circulation (ROSC).

**Methods and analysis** This is a prospective observational study. The participants (n = 60) who achieve ROSC will be distributed into two groups (non-survivor and survivor) based on 28-day survival. Healthy volunteers (n = 30) will be enrolled as controls. Each individual's relevant information will be extracted from Electronic Medical Record System in Xinhua Hospital, including demographic characteristics, clinical data, laboratory findings and so on. On days 1, 3 and 7 after ROSC, blood samples will be drawn and batch tested on the level of serum neuron-specific enolase, soluble protein 100β, L-PGDS, procalcitonin, tumour necrosis factor-alpha and interleukin-6. The cerebral performance category score was assessed on the 28th day after ROSC.

**Ethics and dissemination** This study was performed with the approval of the Clinical Ethical Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval No. XHEC-C-2023-130-1). The results will be published in a peer-reviewed journal.

**Trial registration number** Chinese Clinical Trial Registry (ChiCTR2300078564).

## STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study uses multimodal markers to predict 28-day mortality in cardiac arrest (CA) patients.
⇒ The study was carried out in a large comprehensive teaching hospital, serving a population of 1.2 million, to ensure the credibility of the experimental data.
⇒ The trial is a single-centre, prospective study with a small sample size due to limited time available for enrolling patients.
⇒ Our follow-up period ended 28 days after the restoration of spontaneous circulation, and longer follow-ups went beyond the scope of this trial and thus, we could not establish the relationship between lipocalin-type prostaglandin D synthase and long-term mortality in patients with CA.

## INTRODUCTION

Cardiac arrest (CA), due to its high morbidity and mortality, is one of the major public health problems that seriously threatens the life and health of the people. According to the latest findings, the annual incidence of CA is close to 637 000 persons in the USA.[1 2] Current estimates suggest that the survival rate for CA ranges from 15% to 22%.[3] Among patients successfully resuscitated from CA and treated in the postresuscitation phase, mortality remains high in the intensive care unit (ICU) due to the pathophysiological process of so-called post-cardiac arrest syndrome (PCAS), which is characterised by primary (ischemia) and secondary (reperfusion) injury that affects the brain, lung, heart and kidneys.[4] Moreover, the neurological outcome is one of the main determinants of survival in post-CA patients.

The guidelines now recommend the use of a multi-modal post-CA prognostication that includes four elements: (1) biochemical markers, (2) imaging, (3) clinical examination and (4) electrophysiological investigations.[5 6] So far, despite the availability of these multifaceted diagnostic tools, the predictive value of all these methods remains limited. Therefore, identifying blood biomarkers with higher sensitivity and specificity could help reduce unnecessary expensive treatment costs and uncertainty of the relatives.[7]

Prostaglandin D synthase (PGDS) is mainly divided into two types, namely, lipocalin-type PGDS (L-PGDS) and hematopoietic PGDS.[8] L-PGDS, a member of the lipocalin superfamily, is mainly expressed in the central

nervous system (CNS) and cardiovascular system and can be detected in serum, cerebrospinal fluid (CSF) and urine.[9 10] L-PGDS is involved in various neurological processes, including neuronal apoptosis and clearance of neurotoxic metabolites.[11 12] Previous evidence has shown that L-PGDS$^{-/-}$ mice had significantly greater infarct volume and higher neurological function score thanwild type mice after transient or permanent focal cerebral ischemia.[13]

L-PGDS has been found to be highly expressed in various CNS diseases, such as spinal cord injury, Alzheimer's disease, and transient and permanent ischaemic brain injury.[13 14] At present, increased serum levels of L-PGDS in patients undergoing coronary angiography have been correlated with the severity of stable coronary artery disease.[15] Serum L-PGDS levels increase in association with the progression of renal function worsened in patients with hypertension.[16] However, the role of L-PGDS in patients resuscitated from CA has not been clarified to date. Thus, we postulate that a change in the concentration of L-PGDS among CA survivors could provide a reliable clue for the clinical outcomes.

## OBJECTIVE

The goal of this study was to evaluate the dynamic fluctuations of serum L-PGDS, inflammatory factors, and neurological damage biomarkers and to investigate whether serum L-PGDS can predict the severity and prognosis in CA survivors.

## METHODS AND ANALYSIS

### Design and setting

This is a prospective observational study to be conducted in the emergency ICU in Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Shanghai, China).

During the whole period of this trial, we performed according to the Declaration of Helsinki.[17] The flowchart of the study is illustrated in figure 1, while table 1 summarises study enrolment, interventions and assessments.

### Study participants

Patients (age ≥ 18) who successfully resuscitated from in-hospital or out of hospital CA will be recruited and divided into different groups (survivor group or non-survivor) based on whether they were survived 28 day after restoration of spontaneous circulation (ROSC). Significantly, all enrolled patients were provided scientifically standardised treatment in accordance with the 2020 International Consensus on Cardiopulmonary Resuscitation on admission to the ICU.

### Patients and public involvement

Participants will be consecutively recruited through the recommendation of the attending physician. Patients and the public did not participate in the design or the enrolment and implementation of this study and will not be involved in the dissemination plans of the research results.

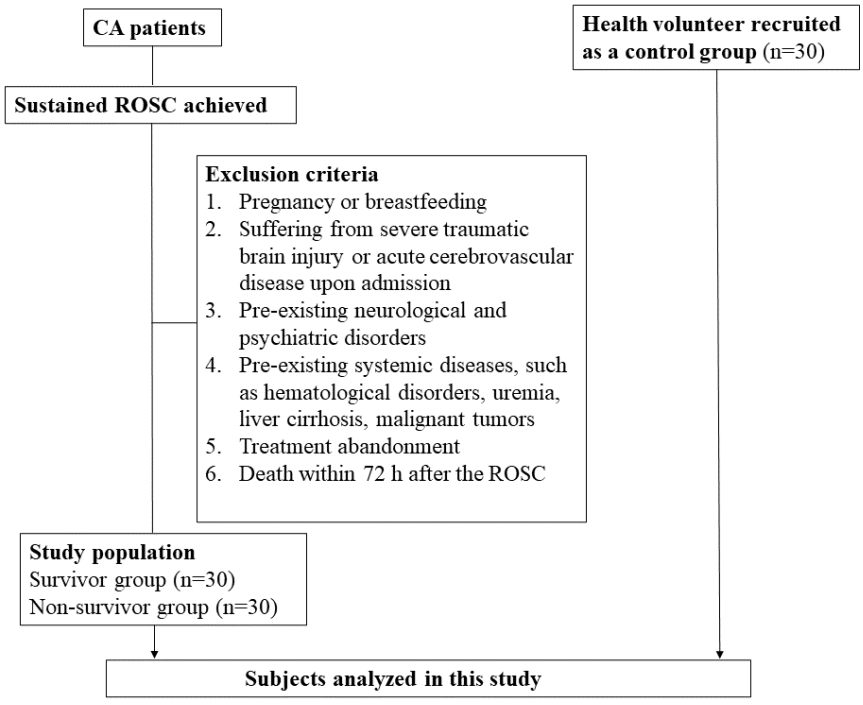


**Figure 1** Flowchart of the study design. Abbreviations: CA, cardiac arrest; ROSC, restoration of spontaneous circulation.

**Table 1** Overview of study visits.

| | Study period | | | | |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 |
| Visit | 0d | ROSC 1d | ROSC 3d | ROSC 7d | ROSC 28d |
| Enrolment Eligibility Screen Informed consent | × × | | | | |
| Medical history Diabetes Hypertension Coronary heart disease Cerebrovascular disease Chronic pulmonary disease Chronic kidney disease | × × × × × × | | | | |
| Demographic data Age Male | × × | | | | |
| CPR time | × | | | | |
| Initial heart rhythm | × | | | | |
| SOFA score | | × | × | × | |
| APACHE II score | | × | × | × | |
| Laboratory indicators Lactate Albumin Total bilirubin Creatinine Haemoglobin White blood cell count Platelet count | | × × × × × × × | × × × × × × × | × × × × × × × | |
| Biomarkers L-PGDS NSE S100β PCT TNF-α IL-6 | | × × × × × × | × × × × × × | × × × × × × | |
| CPC score | | × | × | × | |
| Length of ICU stay | | | | | × |

APACHE II score, Acute Physiology and Chronic Health Evaluation score; CPC score, Cerebral Performance Category score; CPR, cardiopulmonary resuscitation; ICU, intensive care unit; IL-6, interleukin-6; L-PGDS, Lipocalin-type prostaglandin D synthase; NSE, neuron-specific enolase; PCT, procalcitonin; ROSC, restoration of spontaneous circulation; SOFA score, Sepsis-Related Organ Failure score; S100β, soluble protein 100β; TNF-α, tumour necrosis factor-alpha.

## OUTCOMES

### The primary outcome

The primary study endpoint of this study is the change in L-PGDS on days 1, 3 and 7 after ROSC.

### Secondary outcomes

Secondary outcomes include the following：

1. Survival to 28-day after ROSC
2. Cerebral Performance Category (CPC) score
3. Sepsis-Related Organ Failure (SOFA) score
4. Acute Physiology and Chronic Health Evaluation (APACHE II) score.

### Date collection

If the enrolled patients experience multiple CA during hospitalisation, we will collect the first occurrence of CA uniformly. Each individual's relevant information will be extracted from Electronic Medical Record System in Xinhua Hospital by research assistants, including demographic characteristics (eg, age and sex), medical history, clinical data, the length of ICU stay, cardiopulmonary resuscitation (CPR) time, initial heart rhythm as well as laboratory indicators (eg, lactate, albumin, total bilirubin, creatinine, haemoglobin, white blood cell count and platelet count). SOFA score and APACHE II score will be obtained according to relevant scoring standards on the days 1, 3 and 7 after resuscitation, respectively. CPC score on day 28 after ROSC was recorded in order to assess the neurological prognosis. Survival status will be reviewed on day 28 after CA, and patients discharged before this time point will be contacted by phone to determine survival status.

### Blinding

After all the above data collection is completed, the research assistant will organise the data, delete personal identification information such as the patient's hospitalisation number and name and assign a unique number to the patient. The research assistant is blind to the content of this study.

### Measurement of biomarkers

The patients' fresh blood will be obtained from a peripheral vein by nurse on days 1, 3 and 7 after resuscitation or when recruiting healthy volunteers. Blood samples will be centrifuged for 15 min at 3000 rpm at 4°C. Subsequently, separated serum will be evenly divided into cryogenic storage tubes and stored at −80°C until further analyses. L-PGDS (BioAssay), serum neuron-specific enolase (NSE), soluble protein 100β (S100β), procalcitonin (PCT), tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) will be measured using ELISA kits, according to the manufacturer's protocol.

### Sample size estimation

We calculated an effect size of 0.5 (large effect size) based on the results of the relevant literature.[15] The sample size was then calculated using G*Power software (V. 3.1.9.7), assuming $\alpha = 0.05$ , power$(1\text{-}\beta) = 0.95$ was estimated that at least 22 patients would be required for each group. Finally, we chose to include 30 people in each group to prevent drop-outs.

### Statistical analysis

The results are expressed as counts and percentage (%) for categorical variables, continuous variables with a normal distribution are expressed as the mean ± SD, while skewed continuous variables are represented as median (25th–75th percentile). Pearson $\chi^2$ or Fisher exact tests were employed (as appropriate) to compare demographic variables. Repeated-measure analysis of variance will be used to compare the changes in variables

among survivors, non-survivors and healthy volunteers at different time points, and then the Bonferroni test will be used for multiple comparisons. Bivariate correlation analyses using the Spearman correlation coefficient will be conducted to examine the relationship between L-PGDS and other variables. Receiver operating characteristic curves will be drawn and the areas under the curve (AUC) will be calculated to assess the value of serum L-PGDS for predicting 28-day all-cause mortality. The AUCs will be compared using the DeLong's test. The Youden's index will be used to determine the optimal thresholds for assessing sensitivity and specificity. Spearman non-parametric correlation was used for calculating the correlations between L-PGDS and other biomarkers (NSE, S100β, PCT, TNF-α, IL-6, lactate and CPR time).

Data analyses will be conducted with the use of SPSS V. 21.0 software (IBM, Armonk, NY) and R V. 4.3.1 software (R Foundation for Statistical Computing, Vienna, Austria). When two-tailed $p$ value was <0.05, differences are taken to be statistically significant.

## DISCUSSION

As for multi-modal post-CA prognostication, imaging requires patients to be transferred to a specific examination site,and electrophysiological examination requires qualified experts in relevant fields; however, biomarkers as laboratory data are the most convenient and objective indicators with high repeatability, which is beneficial for clinical applications.[18] Over the past few decades, there has been a growing interest in the study of biomarkers. Many investigators have proven that the diagnosis of many diseases is effective, such as severe head injury (NSE, S-100β), myocardial infarction (troponin), renal failure (creatinine), acute pancreatitis (amylase) and malignant diseases (prostate-specific antigen, α- Photoprotein).[19–22] Early and quantitative assessment of the changes in biomarkers has important clinical significance for predicting the prognosis of patients with CA and strengthening supportive treatment in patients after ROSC.

After ROSC, the duration of global ischemia, along with the subsequent systemic inflammatory response syndrome and organ impairment, greatly influence the severity of PCAS.[23 24] In this study, indicators representing the severity of PCAS and organ dysfunction (APACHE II and SOFA scores, NSE and S100β), as well as immune inflammatory response after CA (IL-6 and TNF-α), were included in the detection.[25] L-PGDS, a major brain-derived protein in CSF, is involved in the development and progression of many diseases due to altered expression levels in the brain.[26 27] The possible mechanisms by which L-PGDS affects prognosis are as follows. First, L-PGDS and its product, PGD2, have been shown to exert anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and chemokines, as well as by promoting the resolution of inflammation. Second, L-PGDS has been implicated in neuroprotection, particularly in the context of ischaemic brain injury.[13] This neuroprotective effect may contribute to improved outcomes in patients with CA and subsequent ROSC. Third, L-PGDS, as a member of the lipocalin family, can bind and transport various lipophilic molecules, including retinoids, biliverdin and bilirubin.[28] This function may help to regulate lipid metabolism and reduce the risk of atherosclerosis, thereby affecting prognosis in CA patients.

Therefore, we intend to conduct a prospective observational study in CA patients after ROSC. Our primary objective is to determine the clinical validity of serum L-PGDS for predicting 28-day mortality and assessing severity in patients with CA who attain ROSC. The correlation between L-PGDS and other prognostic biomarkers (including NSE, S100β, PCT, TNF-α, IL-6, lactate and CPR time) currently used for CA will also be analysed. With the resulting evidence, our ultimate goal is to provide useful prognostic information for continuous L-PGDS measurements in patients with CA and the dynamic of L-PGDS may be employed to guide medical decisions to provide patient outcomes in ICU.

There are also some limitations to our study. First, our follow-up period ended on day 28 after the ROSC, and longer follow-ups went beyond the scope of this trial and thus, we could not establish the relationship between L-PGDS and long-term mortality in patients with CA. Second, the limited sample size used in the experiment may not give definitive answers to these questions. However, based on the results of this experiment, we can draw a preliminary conclusion. Prospective multi-centre clinical trials with large sample sizes are required for further study.

## ETHICS AND DISSEMINATION

This study has been approved by the Clinical Ethical Committee of Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Approval No. XHEC-C-2023-130-1). This study presents minimal to no risk to the subjects. In order to avoid publication bias in future meta-analyses, the results will be published regardless of the trial's outcome.

**Contributors** The authors' responsibilities were as follows: conceptualization: HF; drafting the manuscript: HF; implementation of the trial: HF, SW and PX; funding acquisition: XG and SP; supervision: XG and SP; revision of the article: HF, SW, PX, ZF, SP and XG.

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**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

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