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Macrophage-Specific Protein Perforin-2 is Associated with Poor Neurological Recovery and Reduced Survival After Sudden Cardiac Arrest

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Abstract

Background: Biomarkers involved in inflammation and stress response were implicated in patients who were successfully resuscitated from out of hospital cardiac arrest (sR-OHCA). Here we report that macrophage-expressed gene, perforin-2, an evolutionarily conserved protein with membrane attack domain, is associated with poor neurological outcomes and mortality after sR-OHCA.

Objectives: To examine the association between circulating perforin-2 protein measured within 6-hours of sR-OHCA, mortality and neurological outcomes.

Methods: We prospectively enrolled 144 sR-OHCA patients from 4 different tertiary care centers. We measured perforin-2 and other conventional clinical biomarkers and compared between survivors *vs.* non-survivors. The neurological outcomes were dichotomized as *poor* or *good* according to the cereberal performance score.

Results: At the end of the hospital stay, 47% of the patients had poor neurological status, of whom 95% had in-hospital mortality. Serum perforin-2 levels were significantly higher in patients with *poor* neurological status, compared to the ones with *good* neurological recovery (ng/ml, 13.7 ± 45.9 *vs.* 1.2 ± 7.0, p=0.01). There were no differences in other routinely measured biomarkers and left ventricular ejection fraction. On multivariate logistic regression, elevated perforin-2 (OR: 12.78, 95% CI: 1.0–17.8, p=0.02), comatose on presentation (OR: 27.82, 95% CI: 0.2 – 19.5, p=0.02) and non-shockable rhythm were the significant predictors of poor neurological outcome.

Conclusions: This study reports a novel macrophage-expressed circulating biomarker perforin-2 to be strongly associated with reduced survival and poor neurological outcomes in sR-OHCA.

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Conflict of interest: None.

These data can guide clinicians to prognosticate survival and neurological outcomes in sR-OHCA, and also form the basis for future therapeutic approaches.

Subject Terms:

Biomarkers; Mortality; Neurological Recovery; Perforin-2; Sudden Cardiac Arrest

Introduction

Despite increasing awareness on early recognition and bystander resuscitation for out of hospital sudden cardiac arrest (OHCA), only 30% of patients survive to be admitted to the hospital for further management. Approximately 12% of the survivors are discharged from the hospital with only 8% having good neurological recovery[1]. Advances in the care of hospitalized patients after sudden cardiac arrest (SCA) with prompt initiation of hypothermia protocol is proven to preserve or improve the neurological function[2]. However, the long-term prognosis of OHCA is still sobering[3]. The causation for very high mortality and poor neurological outcomes may be multifactorial, including anoxic tissue injury and systemic inflammatory response, among the other factors surrounding the event of OHCA[4].

Prior studies have examined multiple factors including arterial pH, serum lactate, length-of-resuscitation, type of rhythm at the time of arrest, and patient location at the time of event as the predictors of poor outcome[5,6]. We have recently reported a macrophage-expressed circulating biomarker, galectin-3 as a predictor of mortality in the survivors of cardiac arrest[7]. Other groups have reported the association between elevated inflammatory markers including C-reactive protein, interleukin-6, and tumor necrosis factor-alpha, and poor survival after OHCA[8,9]. Despite these survival data, there is limited understanding on the role of serum biomarkers to predict neurological outcomes. The neuronal damage leading to cell death may involve many factors, including excitotoxicity, disrupted calcium homeostasis, free radical formation, pathological protease cascades, and activation of cell death signaling pathways from acute anoxia[10,11]. Studies have implicated that resident macrophage activation after the anoxic event can trigger an inflammatory cascade, leading to mononuclear cell infiltration, cytokine, and complement release, ultimately leading to irreversible neurological damage[11]. However, there were no studies examining specific anoxia-sensitive neurotoxic markers implicated for acute neuronal membrane damage, cytolysis, and progressive loss of neurologic function.

Our previously published studies in a pre-clinical model of acute cardiac ischemia reported a significant overexpression of macrophage expressed inflammatory genes including CD68, galectin-3, and MPEG-1 (Perforin-2).[12] Of the more than 12,000 genomic markers analyzed, we identified perforin-2 as one of the most robustly elevated genes. Perforin-2 is one of the most ancient and highly conserved members of the membrane attack complex (MAC)/perforin-like (PF)/cholesterol-dependent cytolysin (MACPF/CDC) superfamily[13]. Perforin-2 is capable of inducing apoptosis in target cells which is often triggered by $CD4^+$ $CD28^-$ T-cells[14,15]. A recent publication by Pang and associates has demonstrated that perforin-2 is a membranolytic protein primarily activated in acidic cellular microenvironment[13]. Therefore, we have hypothesized that elevated serum perforin-2 can be a surrogate marker of

neurologic injury after anoxic event. In this study, we measure the circulating levels of perforin-2 in successfully resuscitated out of hospital sudden cardiac arrest subjects in relation to neurological status at the hospital discharge and short-term (90-day) survival after OHCA.

Methods

This is a prospective multicenter study which enrolled successfully resuscitated after out of hospital cardiac arrest (sR-OHCA) and admitted for further clinical management to one of the four tertiary care hospitals at Buffalo-Niagara metropolitan area (*Gates Vascular Institute, Buffalo General Medical Center, Sisters of Charity, and South Buffalo Mercy Hospital*) between March 2016 to December 2018. All clinical procedures and protocols conformed to institutional guidelines and were approved by the institutional review board (IRB) at the University at Buffalo.

Patient Enrollment

Inclusion criteria: Patients who presented to the emergency department with sR-OHCA were included in this study. SCA was defined as non-traumatic, unexpected circulatory arrest occurring within 1-hour of the onset of symptoms in an apparently asymptomatic subject following which there was successful return of spontaneous circulation (ROSC). Unwitnessed SCA was considered if the victim was in good health within 24 hours before the event and had successful ROSC after resuscitation. Patients who did not have OHCA but presented with acute coronary syndrome (non-ST elevation myocardial infarction or unstable angina) were recruited as controls (structural heart disease-SHD group) for the comparison. Serum samples from healthy controls without known heart disease were used to establish the baseline perforin-2 levels for comparison.

Exclusion criteria: Patients with a documented trauma preceding SCA, acute myocardial infarction (AMI) or cardiac surgery within the preceding three months were excluded from this study. Patients with active malignancies were also excluded due to the potential confounding effects on biomarkers from the cancer itself or from the ongoing anticancer therapies.

SCA subgroup adjudication.: Patients with a clear non-cardiac etiology of SCA were classified as *non-cardiac SCA.* The remaining patients were adjudicated as presumed or proven SCA of cardiac origin (*cardiac SCA group*). Patients with SCA of cardiac origin and evidence of obstructive CAD based on a prior or current coronary angiogram, a history of prior or current MI, or revascularization were considered as SCA patients with ischemic heart disease (*IHD SCA group*). Patients with cardiac SCA who were proven not to have evidence of obstructive CAD and prior history without MI or revascularization were categorized as SCA patients without IHD (*non-IHD SCA group*). Obstructive CAD was defined as presence of >50% stenosis of the left main, >70% stenosis in a major coronary vessel, or 50–70% stenosis with fractional flow reserve of ≤0.8. *The IHD SCA* group was further sub-classified into who had SCA due to an acute MI (*AMI SCA group*) versus

without acute MI (*non-AMI SCA group*) based on electrocardiography, biomarkers at the time of presentation, and findings of coronary angiography.

Serum perforin-2 levels.: Within the first 6 hours of initial hospital presentation, blood samples were collected from the peripheral veins using 2 ml BD Vacutainer® Heparin tubes that are spray-coated with lithium heparin. Samples collected in these tubes are routinely used for plasma determinations in chemistry. After centrifugation, serum samples were stored in refrigerators at −80°C until perforin-2 assays were performed.

First, a pilot microplate Enzyme-Linked Immunosorbent Assay (ELISA) was performed in five serum samples to determine the optimal assay conditions and develop standard and calibration curves (LSBio, Seattle, WA; Kit#F39300). Consequently, all samples were diluted 10-fold with pre-prepared assay diluent, and each ELISA well was loaded with 100μl of the serum samples or perforin-2 standards. Serum perforin-2 levels were quantified from optical density analysis at 450nm, in relation to pre-determined standard perforin-2 concentrations. The background correction was done in reference to the optical density from the wells that contained sample diluent only. R^2 values were used to determine fitting using linear and semi-log models according to the manufacturer's instructions. The experiments were repeated twice to determine reproducibility.

Data abstraction.: Baseline demographics, clinical characteristics, initial laboratory values, electrocardiographic and coronary angiogram data were collected for all patients by chart review from the time of presentation to the hospital. Serum biomarker levels including at presentation and peak troponin-I levels and creatine kinase-MB (CK-MB) were obtained from clinical chart review from the samples collected during the hospitalization as a part of their standard of care. Other clinical factors surrounding the SCA event which could have significant impact on mortality such as length of cardiopulmonary resuscitation (CPR) time to ROSC and presence of a witness at the time of the event were recorded for further analysis.

Follow-up and neurological outcomes.: The primary outcome was neurological recovery at the end of hospital stay. Neurological status at the end of hospital was assessed using the cerebral performance category (CPC), which is a well-validated prognostic algorithm commonly used in clinical practice[16,17]. CPC levels 1 (good recovery) and 2 (moderate disability) were considered as favorable neurological outcome, whereas CPC levels 3 (severe disability), 4 (vegetative state) and 5 (death) were defined as unfavorable outcome[5,6]. Secondary outcome was all-cause mortality at 90 days from the time of OHCA. All-cause mortality was obtained from medical chart review, telephone contact with family members, and/or primary care physician.

Statistical analyses.: Data are expressed as mean ± standard deviation and percentage (%) for continuous and discrete variables, respectively. A Chi-square (χ^2) test or Fisher exact test and Mann-Whitney U or student t-tests were used to compare categorical and continuous variables, respectively as appropriate. Correlations between variables were analyzed using Spearman's correlation. Univariate logistic regression was performed to establish the predictors of poor neurological outcomes. Variables that were significant in

univariate logistic regression analysis were then used in multivariate logistic regression analysis. Before performing multivariate analysis, assumption of proportionality was assessed. The odds ratio (OR) and 95% confidence interval (CI) were calculated for each independent variable.

To assess the ability of independent variables to predict poor neurological outcome, receiver operating characteristic (ROC) curves were generated for each variable that was significant on a multivariate logistic regression analysis. For markers with a significant AUC, optimal cut-points were chosen at the maximized Youden index value for predicting all-cause mortality. All alpha levels were set at 0.05 and analyses were performed using JMP version 14 (SAS Inc.).

Results

Baseline characteristics.

This prospective multicenter study recruited a total of 193 subjects [OHCA: 144; SHD: 20; healthy controls: 29]. We have summarized the baseline demographics, clincal characterstics and outcomes in Table 1. Female sex, family history of SCA, prior MI and revascularization, and use of aspirin were more common in SHD group. In comparison to healthy controls and those with SHD, patients with OHCA had significantly elevated serum perforin-2 levels (ng/ml: 0.0 ± 0.0, 0.0 ± 0.0 and 6.87 ± 31.99 respectively, $p<0.01$, all comparisons). QTc interval was slightly prolonged in OHCA group compared to SHD group (OHCA: 446.0 ± 53.6ms; SHD: 409.4 ± 40.6ms, $p<0.01$) though the intervals were within normal range in both groups. Similarly, the all-cause in-hospital mortality was significantly higher in OHCA group (45% in OHCA group vs. 5.6% in SHD group, $p<0.01$). All patient demographics, clinical characteristics, laboratory, echocardiographic and event variables around the SCA along with the comparative analysis between those with poor (poor neuro-status group) and good (good neuro-status group) neurological functional recovery at the time of hospital discharge and survivals at 90-day follow-up are shown in Table 2 and 3, respectively.

Overall survival and neurological recovery.

Overall, there was 45% in-hospital mortality and 57% had died at 90-day follow-up. Of those who were surviving at hospital discharge, 87% had good neurological status and the remaining 13% had poor neurological status. Compared to those with good neurological recovery, those with poor neurological recovery had higher prevalence of DM, lower hemoglobin levels, poor GFR, higher serum creatinine, higher BNP level and longer QTc interval. These patients with poor neurological recovery were also more frequently on a diuretic therapy, comatose at presentation, non-shockable rhythm at the time of OHCA and underwent targeted temperature therapy during hospitalization.

Biomarker comparisons in OHCA with poor vs. good neurological recovery.

Compared to good neurological recovery group, poor neurological recovery group had significantly elevated perforin-2 (ng/ml, good neuro-status: 1.2 ± 7.0; poor neuro-status: 13.7 ± 45.9, $p<0.01$) and serum BNP (pg/ml, good neuro-status: 363.1 ± 634.8; poor neuro-

status: 1181.3 ± 1544.0, p<0.01) levels. However, there were no differences in the initial or peak troponin, creatine kinase (CK), and CK-MB levels.

Prognostic significance of biomarkers.

Univariate logistic regression analysis for predictors of poor neurological recovery is shown in Supplemental Table 1. The significant variables were then included in multivariable logistic regression analysis for poor neurological recovery, which showed elevated perforin-2, shockable rhythm on presentation, and comatose state after obtaining ROSC remained independent predictors for poor neurological outcomes (Supplemental Table 1).

Patients who had elevated perforin-2 levels (OR: 12.78, 95% CI: 1.0–17.8, p=0.02), comatose on presentation after OHCA (OR: 27.82, 95% CI: 0.2 – 19.5, p=0.02) and initial non-shockable rhythm (OR: 17.04, 95% CI: 0.7–15.7, p=0.01) had higher odds of poor neurological outcomes. Univariate Cox proportional hazard analysis for predictors of survival at 90-day follow-up are shown in Supplemental Table 2. The significant variables were then included in multivariate Cox proportional hazard analysis for predictors of survival, which showed perforin-2, BNP and initial shockable (VT/VF) rhythm as significant predictors for survival at 90 days (Supplemental Table 2). The Kaplan-Meir survival curves at 90 days for significant variables perforin-2, shockable rhythm, and BNP are shown in figures 1, 2 and 3, respectively. We chose a perforin-2 level of 8.8 ng/ml (sensitivity: 0.18, specificity: 0.96) and BNP level of 173pg/ml (sensitivity: 0.65, specificity: 0.66) based on the maximized Youden index obtained by ROC curves. This demonstrated that patients with OHCA with perforin-2 levels of more than 8.8 ng/ml (OR: 5.27, 95% CI: 1.10 – 25.08, p=0.03), BNP levels of more than 173pg/ml (OR: 5.93, 95% CI: 1.96–17.91, p<0.01) and non-shockable rhythm (OR: 7.40, 95% CI: 3.11–17.63, p<0.01) had significantly higher odds of death at 90-day follow-up.

OHCA subgroup analysis.

Of the 144 patients with OHCA, 69% patients were of cardiac SCA and 31% of non-cardiac SCA. Of those with cardiac SCA, 38% had AMI as a cause of OHCA. SCA patients were found to have on average 7-fold increase of serum perforin-2 levels compared to healthy controls or patients with SHD (OHCA, 6.87±31.99 ng/ml; SHD/Healthy control: 0.0 ± 0.0 ng/ml, p<0.01, all comparisons). Among cardiac SCA, there was no difference in perforin-2 level among patients with IHD SCA vs. non-IHD SCA (IHD SCA: 5.57 ± 28.74; Non-IHD SCA: 9.25 ± 36.74, p:0.44) but was significantly elevated in non-survivors compared to survivors as shown in Supplemental Table 3.

Discussion

This is the first study showing elevated serum levels of perforin-2, a pore-forming protein containing the membrane attack complex domain, as a predictor of adverse neurological outcomes in the survivors of cardiac arrest. Perforin-2 is expressed by mature macrophages that are active and able to perform their immune functions including antigen presenetation, chemotaxis and cytokine release[18]. Our prior studies in a preclinical murine model of ischemic cardimyopathy showed elevated perforin-2 gene expression[19]. This study expands

on our previous data reporting the utility of this novel protein in adjudicating patients with worse neurological outcomes after cardiac arrest.

Pore-forming proteins are reminiscent of the ancient innate immune system that was required to remove the microbial agents. The conservation of these proteins in mammals represents a critical functionality of this protein. In humans, perforin-2 is expressed by mature macrophages. In non-macrophage cells, perforin-2 is not expressed under basal condition but can be induced with pro-inflammatory agents including different types of interferons. More recently, perforin-2 is reported to be expressed under the acidic microenvironment[13], which is expected in patients with circulatory failure resulting from SCA.

Macrophages have been implicated in the heart-failure-related mortality. The inflammatory mediators secreted by macrophages including IL-1, tumor necrosis factor-α, galectin-3 and CRP are suggestive of ongoing inflammation as a potential trigger mechanisms of cardiac arrest and potental macrophage-inhibitory approaches were previously advocated[20]. This is the first report identifying a potentially pathogenic molecule of the innate immune system to predict the neurological prognosis in patients who are succesfully resuscitated. The current understanding on the prognostication of neurological outcomes due to severe post-anoxic brain injury is limited. This has led to major clinical challenges on whether to continue life-saving therapies with goals to recovery or withhold treatment. Availability of specific biomarkers will help early adjudication of such patients and better define both short- and long-term therapeutic goals.

Prior studies reported the use of creatine kinase-BB, neuron specific enolase, S-100 protein, myelin basic protein, glial fibrillary acidic protein and IL-8 as the markers of neuronal injury[21–24]. Unfortunately, these neuron-derived biomarkers varied broadly based on the concomitant use of sedation, time of assay from the event, unstandardized assay methods, and post-cardiac arrest hemolysis and had limited prognostic utility[25,26].

Perforin-2 is a highly conserved and central component of innate immune system[27–29]. Under the physiological conditions, perforin-2 plays an important role for the survival of neurones by guarding against external pathogens and lack of perforin-2 may result in the host's inability to clear pathogens[29]. In the presence of neuronal ischemia after cardiac arrest, a widespread expression of perforin-2 could trigger acute inflammation, membrane lysis and death. In addition to its important role in innate immunity, perforins are implicted to regulate the blood brain barrier (BBB)[30]. Cardiac insult with oxidative injury is found to be associated with loss of tight junction protein mRNA expression[31]. After cardiac arrest, hypoxic injury can lead to loss of tight junction proteins and barrier functions and disruption of BBB. This can lead to increased permeability, transvasation of inflammatory cytokines and damage to the central nervous system. For the definite understanding of the intermediary role of perforin-2 in post-resuscitation brain, genetically engineered models with perforin-2 loss and gain-of-function are needed. Since global perforin-2 knockout limits survival, newer CRISPR/Cas9-based approach for brain-specific disruption of perforin-2 can address causation. Further evaluation of the neuronal brain tissue from these models will help

confirm this hypothesis and to design potential drugs that can limit the expression of perforin-2.

Our study also demonstrates that being comatose on presentation and presence of non-shockable rhythm as the predictors of poor neurological outcome. Being comatose and non-shockable rhythm on presentation suggest these patients likely have prolonged anoxic damage and more acidic mileu in the brain that enhance the expression of perforin-2. Importantly, perforin-2 enhanced the predictive utility when combined with other traditional clinical variables. Conventional variables such as troponin I and CPK were not predictive of neurological outcomes; whereas galectin-3 showed the predictive role only in the univariate analysis. Therefore, serum perforin-2 levels at the time of presentation in OHCA provides a novel approach for neurological risk stratification and to individualize medical and device therapy.

Study limitations:

This study has only a modest sample size and follow-up duration as described above. The majority of the patient population was Caucasian, although we attempted to include diverse patient population from multiple centers. Furthermore, one might point to the other variables that can affect survival in OHCA, including the timing of resuscitation, presence of anoxic encephalopathy and initiation of hypothermia protocol. Nevertheless, even after the adjustment for majority of biomarkers and clinical variables, an elevated perforin-2 level remains as a significant predictor of neurological outcome in individuals with OHCA. Predictive role of perforin-2 is further enhanced when other clinical variables are concomitantly included, which may help in further risk stratification and guide management in these patients. Although this study was not initially powered to study gender-specific differences, both uni- and multivariate analyses did not demonstrate gender related differences in biomarker levels or adverse clinical outcomes.

Conclusion:

Elevated perforin-2 level is associated with poor neurological outcomes in resuscitated patients after OHCA. When combined with other clinical variables, comatose on presentation, and presense of non-shockable rhythm, perforin-2 further enhances the risk-stratification after OHCA. We expect that these findings will help clinicians in selecting optimal and individualized management strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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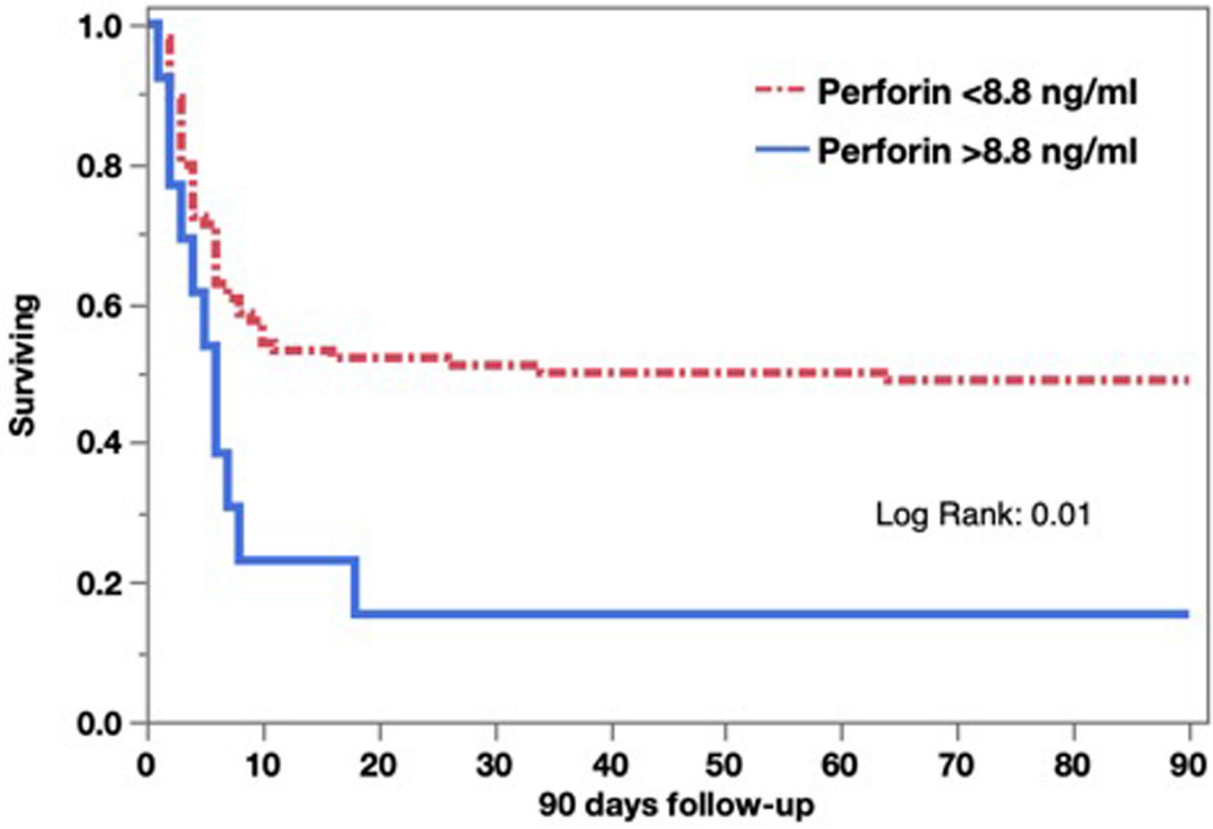

Figure 1. Analysis of all-cause mortality in relation to circulating perforin-2 levels using Kaplan-Meier survival plot.

Elevated serum perforin-2 at presentation in excess of 8.8 ng/ml was associated with reduced survival on 90-day follow up (intergroup comparison by log rank test, p=0.01).

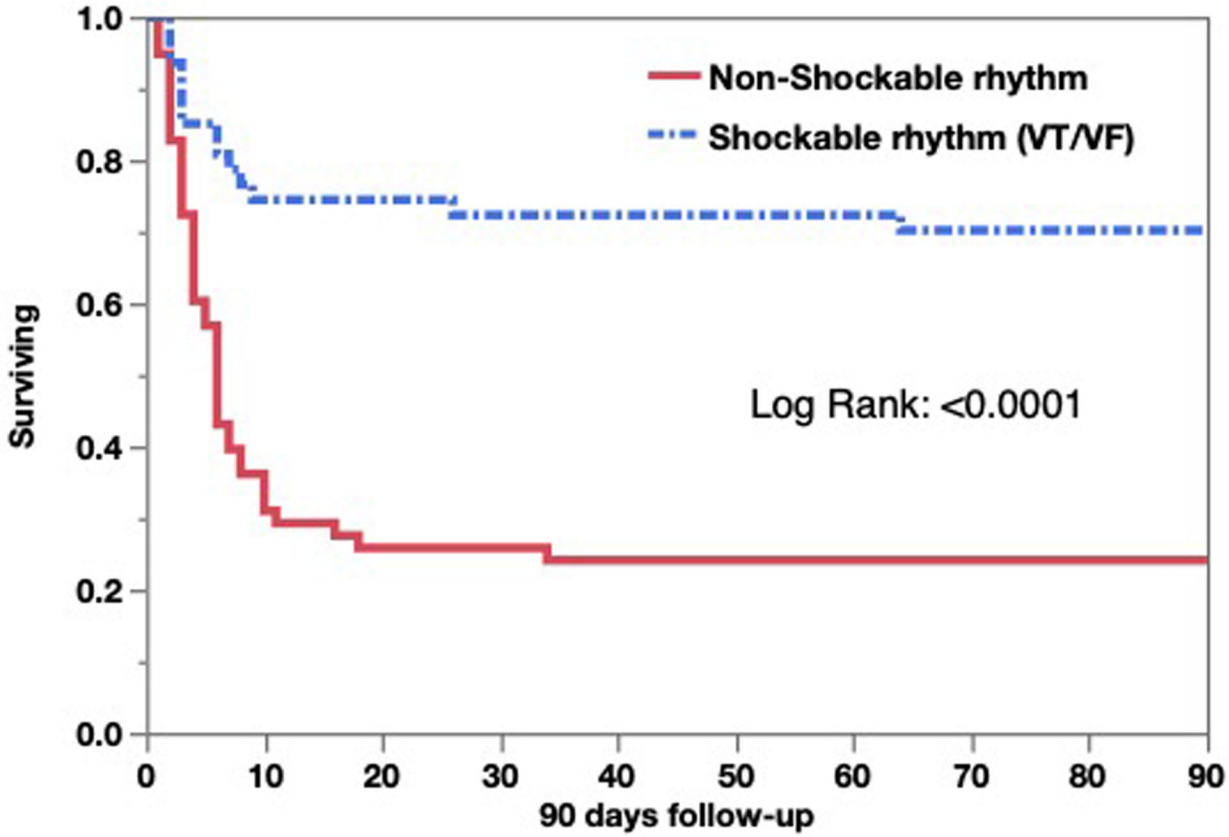

Figure 2. Kaplan-Meir survival plot in relation to shockable rhythm at presentation.

Presence of shockable rhythm (ventricular tachycardia or fibrillation) on cardiac monitor at the time of presentation was associated with higher rate of 90-day survival (log rank $p<0.01$).

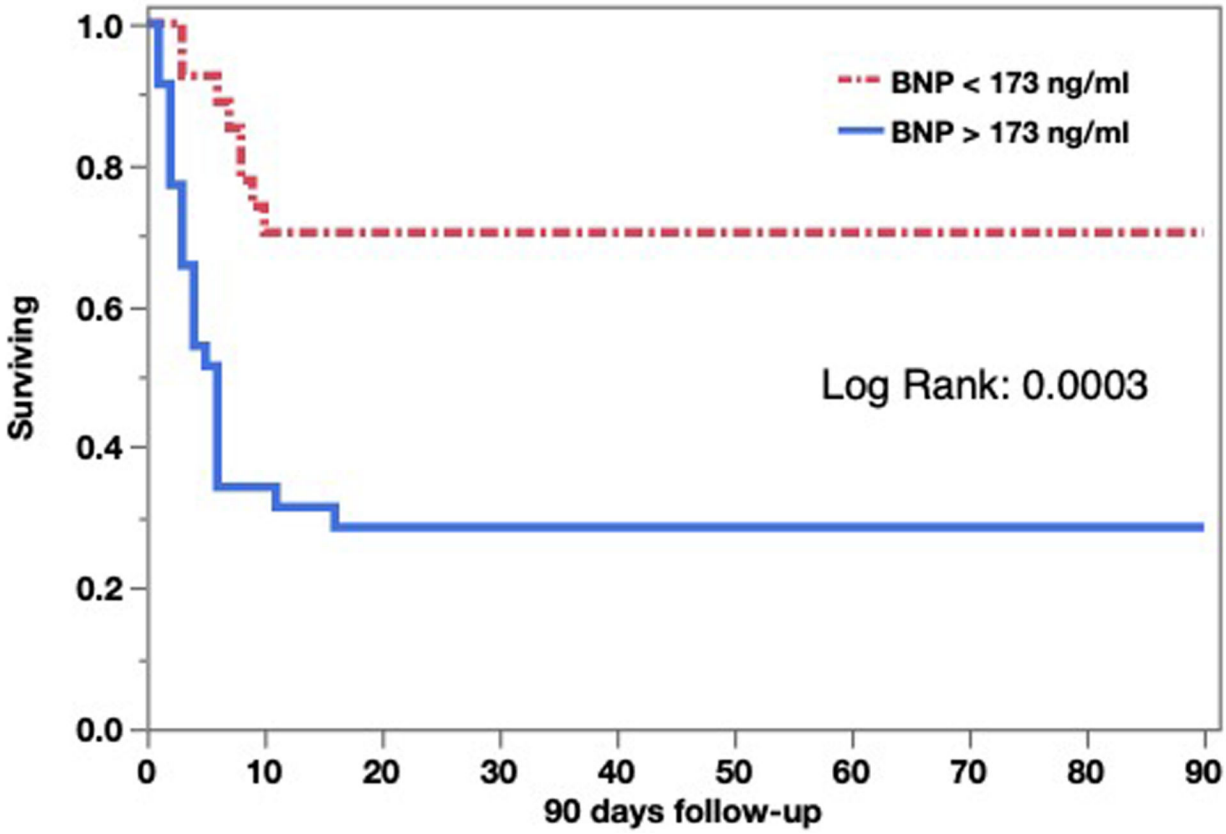

Figure 3. Kaplan-Meier survival plot in relation to elevated BNP levels:
Elevated BNP levels in excess of >173 ng/ml related to reduced 90-day survival (log rank $p<0.01$). BNP, brain natriuretic peptide.

Table 1.

Demographic and baseline characteristics of the study subjects with structural heart disease without arrhythmic events, and resuscitated out-of-hospital sudden cardiac arrest patients

Baseline Characteristics	Structural heart diseases without OHCA (n=20)	OHCA (n=144)	p-value
Demographics			
Age, mean (SD), years	65 ± 13	60 ± 15	0.28
Gender, % females	66.6	36.6	***0.04***
Race, % Caucasians	66.8	85.2	***<0.01***
Risk Factors			
HTN, %	77.8	59.3	0.19
DM, %	22.2	30.0	0.59
CKD, %	0	18.6	***0.04***
CHF, %	33.3	22.8	0.37
Smoking, %	50	51.8	1.0
Atrial fibrillation, %	22.2	11.4	0.25
Family history of SCA	11.1	0.7	***0.03***
Prior Myocardial infarction/revascularization, %	44.4	20.4	***0.04***
Medications			
ASA, %	66.7	29.3	***<0.01***
Statin, %	55.6	42.2	0.31
Beta Blocker, %	61.1	39.3	0.13
Aldosterone blocker, %	0.0	3.6	1.00
ACEi/ARB, %	50.0	32.9	0.18
Diuretics, %	16.7	25.9	0.56
Laboratory values			
Hemoglobin, g/dl	13.3 ± 1.5	12.9 ± 2.3	0.47
Creatinine, mg/dl	1.0 ± 0.3	1.9 ± 1.9	***<0.01***
eGFR. ml/min/1.73m^2	56.4 ± 5.7	48.0 ± 20.4	***0.02***
BNP, ng/ml	259.6 ± 246.1	695.6 ± 1213.8	0.47
Initial CK, U/L	448.0 ± 538.6	1121.5 ± 1649.2	0.10
CK-MB, U/L	76.6 ± 151.9	95.1 ± 218.3	0.90
Initial Troponin, ng/ml	2.2 ± 5.9	1.5 ± 5.8	***0.02***
Peak Troponin, ng/ml	8.2 ± 11.7	27.9 ± 114.0	0.73
Perforin-2, ng/ml	0.0 ± 0	6.87 ± 31.99	**0.01**
Echo and ECG parameters			
LVEF post arrest, %	50.2 ± 12.3	48.1 ± 14.1	0.62
LVEF <35%, %	11.8	17.0	0.36
QTc on initial ECG, ms	409.4 ± 40.6	446.0 ± 53.6	***<0.01***
Outcomes			
All-cause Mortality, %	5.6	45.1	***<0.01***

HTN, hypertension; DM, diabetes mellitus; CHF, congestive heart failure; CKD, chronic kidney disease; SCA, sudden cardiac arrest; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BNP, brain-natriuretic peptide; CK, creatinine kinase; LVEF, left ventricular ejection fraction; and OHCA, out-of-hospital sudden cardiac arrest.

Table 2.

Comparison of the demographic and baseline characteristics of the poor versus good neurological outcomes in resuscitated out of hospital sudden cardiac arrest subjects

	All OHCA (n=144)	Poor Neurological Outcomes (n=66)	Good Neurological Outcomes (n=63)	p-value
Baseline Characteristics				
Age, mean (SD), years	60 ± 15	58 ± 14	62 ± 15	0.07
Female gender, %	36.4	37.8	34.2	0.85
Caucasian, %	83.7	77.2	90.4	0.09
Risk Factors				
HTN, %	60.4	62.1	58.7	0.72
DM, %	30.2	42.4	17.4	***<0.01***
Smoking, %	53.9	56.9	50.7	0.59
CHF, %	24.4	31.2	17.4	0.09
CKD, %	19.3	25.7	12.7	0.07
Atrial fibrillation, %	11.6	12.1	11.1	1.00
Prior MI/Revascularization, %	18.6	16.6	20.6	0.6
Family history of Sudden cardiac death, %	3.1	3.0	3.1	1.00
Medications on presentation				
ASA, %	31.7	34.8	28.5	0.45
Statin, %	44.1	53.0	34.9	0.05
Beta Blocker, %	41.8	48.4	34.9	0.15
ACEi/ARB, %	35.6	34.8	36.5	0.85
Diuretic, %	27.3	40.9	12.9	***<0.01***
Laboratory values				
Hemoglobin, g/dl	12.9 ± 2.3	12.0 ± 2.4	13.6 ± 1.8	***<0.01***
Creatinine, mg/dl	1.9 ± 1.9	2.3 ± 2.5	1.5 ± 1.1	***0.03***
eGFR, ml/min/1.73m^2	48.0 ± 20.4	42.3 ± 21.3	50.7 ± 17.2	***0.02***
BNP, pg/ml	695.6 ± 1213.8	1181.3 ± 1544.0	363.1 ± 634.8	***<0.01***
CK, U/L	1121.5 ± 1649.2	1158.7 ± 1827.0	1162.3 ± 1541.0	0.72
CK-MB, ng/ml	95.1 ± 218.3	73.4 ± 150.4	116.9 ± 264.9	0.10
Initial Troponin, ng/ml	1.5 ± 5.8	1.6 ± 5.5	1.5 ± 6.4	0.89
Peak troponin, ng/ml	27.9 ± 114.0	37.9 ± 159.6	21.3 ± 45.9	0.3
Perforin-2, ng/ml	6.87 ± 31.99	13.7 ± 45.9	1.2 ± 7.0	***<0.01***
Time from event to sample drawn, median, minutes	63	52	66	0.30
Echo- and electrocardiographic parameters				
LVEF post-arrest, %	51.8 ± 13.4	50. 1 ± 14.9	52.2 ± 12.6	0.67
LVEF, <35%, %	17.0	23.3	14.52	0.25
QTc on initial EKG, ms	446.0 ± 53.6	453.9 ± 50.3	431.3 ± 46.5	***0.01***
Event variables				

	All OHCA (n=144)	Poor Neurological Outcomes (n=66)	Good Neurological Outcomes (n=63)	p-value
Witnessed SCA, %	87.1	80.9 (n=63)	93.4 (n=61)	0.05
Bystander CPR, %	95.2	93.7 (n=64)	96.8 (n=63)	0.67
Location of SCA at Home, %	50.3	46.8 (n=64)	53.9 (n=63)	0.47
Comatose, %	74.8	95.3 (64)	53.9 (n=63)	***<0.01***
Initial VT/VF shockable rhythm, %	48.0	23.0	74.1	***<0.01***
Initial Asystole/PEA rhythm, %	40.9	67.6	12.9	
Targeted temperature management, %	38.5	48 (n=64)	29 (n=63)	***0.02***

HTN, hypertension; DM, diabetes mellitus; CHF, congestive heart failure; CKD, chronic kidney disease; SCA, sudden cardiac arrest; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BNP, brain-natriuretic peptide; CK, creatinine kinase; LVEF, left ventricular ejection fraction; SCA, sudden cardiac arrest; CPR, cardiopulmonary resuscitation; VT/VF, ventricular tachycardia/ventricular fibrillation; PEA, pulseless electrical activity, and OHCA, out-of-hospital sudden cardiac arrest.

Table 3.

Comparison of the demographic and baseline characteristics in the survivors and non-survivors of the resuscitated out of hospital sudden cardiac arrest subjects at 90-day follow-up

	All OHCA (n=144)	Survivors (n=79)	Non-survivors (n=65)	p-value
Baseline Characteristics				
Age, mean (SD), years	60 ± 15	59 ± 15	62 ± 15	0.19
Female gender, %	39.3	39.6	38.9	1.00
Caucasian, %	82.2	87.5	77.9	0.21
Risk Factors				
HTN, %	61.9	63.8	60.3	0.84
DM, %	34.3	25.5	41.4	0.10
Smoking, %	52.9	48.9	56.1	0.55
CHF, %	23.3	19.2	26.8	0.48
CKD, %	23.8	19.2	27.6	0.36
Atrial fibrillation, %	11.4	10.6	12.1	1.00
Prior MI/Revascularization, %	19.1	21.3	17.2	0.62
Family history of Sudden cardiac death, %	3.1	5.5	0	0.12
Medications on presentation				
ASA, %	34.3	40.4	29.3	0.30
Statin, %	49.5	42.6	55.2	0.24
Beta Blocker, %	44.8	46.8	43.1	0.84
ACEi/ARB, %	38.1	40.4	36.2	0.69
Diuretic, %	29.8	15.2	41.4	***<0.01***
Aldosterone blocker, %	4.8	2.1	6.9	0.37
Laboratory values				
Hemoglobin, g/dl	12.9 ± 2.3	13.2 ± 2.1	11.9 ± 2.4	***<0.01***
Creatinine, mg/dl	1.7 ± 1.9	1.9 ± 2.4	2.2 ± 1.9	0.48
eGFR, ml/min/1.73m^2	48.1 ± 20.3	48.8 ± 19.3	42.6 ± 21.9	0.13
BNP, pg/ml	738.2 ± 1214.4	470.7 ± 746.6	1118.9 ± 1630.6	0.05
CK, U/L	1121.2 ± 1641.0	1386.8 ± 1785.8	1140.8 ± 1902.5	0.56
CK-MB, ng/ml	95.0 ± 216.9	165.4 ± 318.5	54.7 ± 137.2	0.07
Initial Troponin, ng/ml	1.5 ± 5.7	1.6 ± 7.1	1.4 ± 5.3	0.87
Peak troponin, ng/ml	27.8 ± 113.2	21.3 ± 47.2	36.1 ± 168.7	0.56
Perforin-2, ng/ml	6.9 ± 31.9	1.45 ± 7.1	14.9 ± 48.4	***0.02***
Echo- and electrocardiographic parameters				
LVEF post-arrest, %	51.9 ± 13.4	51.7 ± 12.3	51.5 ± 15.3	0.95
LVEF, <35%, %	18	15	21	0.44
QTc on initial EKG, ms	444.2 ± 48.9	427.3 ± 44.6	457.5 ± 51.9	***<0.01***
Event variables				
Witnessed SCA, %	85.3	93.3	78.9	0.05

	All OHCA (n=144)	Survivors (n=79)	Non-survivors (n=65)	p-value
Bystander CPR, %	95.2	97.9	92.9	0.37
Location of SCA at Home, %	45.6	44.7	46.4	1.00
Length of CPR, mins	17.1 ± 16.8	10.8 ± 8.8	21.2 ± 20.9	***0.01***
Initial VT/VF shockable rhythm, %	44.8	70.2	24.1	***<0.01***
Initial Asystole/PEA rhythm, %	55.2	29.8	75.9	
Comatose, %	78.6	57.5	96.4	***<0.01***
Targeted temperature management, %	38.8	25.5	50.0	***0.01***

HTN, hypertension; DM, diabetes mellitus; CHF, congestive heart failure; CKD, chronic kidney disease; SCA, sudden cardiac arrest; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; BNP, brain-natriuretic peptide; CK, creatinine kinase; LVEF, left ventricular ejection fraction; SCA, sudden cardiac arrest; CPR, cardiopulmonary resuscitation; VT/VF, ventricular tachycardia/ventricular fibrillation; PEA, pulseless electrical activity, and OHCA, out-of-hospital sudden cardiac arrest.