Resistin—Can it be a new early marker for prognosis in patients who survive after a cardiac arrest? A pilot study

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Abstract

Aim

The aim of our study was to evaluate the potential role of resistin in estimating the 30 days prognosis in patients with hypoxic-ischemic organ injury who survived after a cardiac arrest (CA).

Materials and methods

The study included 40 patients resuscitated after a non-traumatic out-of-hospital CA admitted in Emergency Department (ED). All patients were followed for 30 days after CA or until death. Clinical data on admission were recorded. Blood samples were collected on admission in ED (0-time interval), and at 6, 12, 24, 48- and 72-hours following resuscitation. Serum concentrations of resistin, S100B and neuron specific enolase (NSE) were measured. Several predictive scores for the mortality at 30 days were created with logistic regressions.

Results

At each time interval, median serum levels of resistin and S100 B were significantly higher in non-survivors compared to survivors. For NSE, plasma levels were significantly lower in survivors as compared to non-survivors at 48 and 72 hours, respectively. Accurate predictive scores for 30-days mortality were the ones which included the values of resistin and S100B measured at 12 hours after admittance [AUC 0.938 (0.813–0.989), sensitivity 85.71% (67.3%–96%), specificity 91.67% (61.5%‘99.8%), p<0.001], which included the values of all three markers measured at 12 hours after admittance [AUC 0.955 (0.839–0.995), sensitivity 82.14% (63.1%‘93.9%), specificity 100.00% (73.5%‘100.0%), p<0.001] and the that included the values of resistin and S-100B at 6 hours together with serum lactate on
admission [AUC = 0.994 (0.901–1.0), sensitivity 96.4% (81.7%–99.9%), specificity 100.00% (73.5%–100.0%), p<0.001].

Conclusion
In our study, serum levels of resistin or a combination of resistin with S-100B or resistin with S-100B and lactate, were highly predictive for 30 days mortality in resuscitated patients after CA. Further studies on large number of patients are needed to confirm our data.

Introduction
At present, hypoxic-ischemic organ injury after cardiac arrest (CA) remains a major health problem due to increased mortality, morbidity and length of hospital stay. Most of the hospital stay is spent in intensive care units, which leads to increased costs and implications for health system, patient and family. Majority of these patients have poor outcomes. Comatose patients with brain injury after out-of-hospital cardiac arrest (OHCA) have survival rate of approximately 30% [1–3].

Therefore, numerous attempts have been made to identify those patients with high chances of favorable outcome after return of spontaneous circulation (ROSC). In these cases efforts should be made to obtain full recovery. Thus, in the past years a number of studies focused on the potential role of biomarkers, along with clinical and imagistic criteria in estimating outcomes in patients who suffer an out-of-/in-hospital CA [4–8].

Neuron specific enolase (NSE), isomeric form of glycolytic enolase, is found almost exclusively in neurons and neuroendocrine cells. It is now accepted that increased levels of NSE in patients with hypoxic encephalopathy after CA are especially correlated with the severity of neurological injuries and less with the risk of death (the current recommendations of the guidelines are to make it a prognostic marker after the first 3 days of CA) [1–3, 9–11].

S100 protein family has an important regulatory role in different cellular processes. It has been shown that S-100B has a high sensitivity in detecting brain damages, but it can also be released from other injured sources such as adipocytes or myocardial cells [1, 9]. Increased plasma levels of S-100B protein were well correlated with the severity of post-anoxic neurological damages and with unfavorable outcome in patients with cerebral hypoxic injuries [2, 6, 9, 12, 13].

At the moment, it is not possible to correctly predict patients’ outcome after ROSC strictly based on these biomarkers due to their increased heterogeneity and variability in time [3, 8].

Resistin (ADSF- adipose tissue-specific secretory factor) is a protein belonging to resistin-like molecules family and is expressed in humans by a number of cells including adipocytes, peripheral blood mononuclear cells, macrophages and bone marrow cells [14, 15]. Recent studies have drawn attention on the potential role of resistin as a biomarker than can predict mortality in patients with cardio-vascular disease (in close connection with the ability of resistin to influence glucose and insulin metabolism, thrombosis, angiogenesis and smooth muscle cell dysfunction, but as a factor regulating expression of Vascular Cell Adhesion Molecule-1 on endothelial cells) and as a marker of inflammation that can predict survival in critically ill patients. At this point, this correlation was frequently associated with organ dysfunction in patients with sepsis [16–19], but its role in patients who have a successfully resuscitated CA (although in this case, inflammatory processes underlie hypoxic organs injury) is unknown.
The aim of our study was to evaluate the potential role of resistin in estimating 30 days prognosis in patients with hypoxic-ischemic organ injury who survived after a CA.

Materials and methods

The study was prospective, analytical, longitudinal, observational and cohort type. It included all consecutive patients resuscitated after non-traumatic OHCA admitted to the Emergency Department (ED) of County Emergency University Hospital Cluj-Napoca, who were subsequently transferred in intensive care units, between May 2016 and October 2017. All patients were followed for 30 days after CA or until death. This study was approved by the Ethics Committee of 'Iuliu Hațieganu' University of Medicine and Pharmacy, with registration number 59/14.03.2016. Informed consent for inclusion in the study was obtained from patients’ proxies in all cases. Inclusion criteria were age between 18–85 years and CA. Exclusion criteria were age under 18 and over 85 years, pregnancy, CA due to trauma, acute bleeding from non-traumatic condition, re-arrest with unsuccessful resuscitation within 6 hours from hospital arrival, arrest secondary to hypothermia, terminal neoplastic disease, inmates and absence of informed consent.

Study protocol

CA was managed in all patients according to European Resuscitation Council Guidelines 2015. Resuscitation was initiated on-site by emergency medical team members [20, 21]. All the study patients were treated with conventional cardiopulmonary resuscitation (CPR). Extracorporeal cardiopulmonary resuscitation (ECPR) has not been applied to any patient in our study. Fluid infusion and vasopressors/inotropes, alone or in combination (based on individual clinician judgment) were administered to maintain mean arterial pressure $\geq 65$ mmHg and urine output of $\geq 0.5$ ml/kg/hour. After successful resuscitation, for comatose patients that were able to maintain a systolic blood pressure above 90 mmHg (mean arterial pressure—MAP $\geq 65$ mmHg, with or without vasopressors) and did not present with sepsis, controlled therapeutic hypothermia was administered in the first 24 hours. A core temperature between 34–35˚C was achieved using ice bags and cooling blankets. Reheating was slow at a rate of 0.25–0.5˚C/hour. Hyperthermia, convulsions and hyperglycemia were avoided and treated according to current resuscitation protocols [3, 22].

Post-CA shock was defined as need for continuous vasopressors/inotropes infusion to maintain MAP $> 65$ mmHg despite adequate fluid loading, for more than 6 hours after obtaining ROSC. The cause of death was defined as related to post-CA shock (death occurred as a direct consequence of shock, including subsequent multiorgan failure), to hypoxic-ischemic brain injury or to sepsis.

Clinical data such as initial Sequential Organ Failure Assessment (SOFA) score on admission to ED [23], highest SOFA (highest score registered every 24 hours after admission for the first 3 days or until death), age, gender, primary rhythm of CA, duration of resuscitation, the presence or absence of cardiovascular and non-cardiovascular comorbidities were registered.

Blood samples and laboratory assays

Blood samples were drawn from a separate peripheral vein at admission in ED (0-time interval), and at 6, 12, 24, 48- and 72-hours following resuscitation. To collect blood samples, 5 ml serum separator clot activator biochemistry vacutainers were used. The hemolyzed samples were excluded and blood samples were immediately repeated. Samples were centrifuged at 3000 rotation/minutes during the first 60 minutes after prelevation and were stored at -70˚C. Subsequently, serum concentrations of biomarkers resistin, S-100B and NSE were determined.
using a quantitative sandwich immunoassay technique (ELISA; BioVendor, LM, Czech Republic) according to the manufacturer’s instructions.

**Statistical analysis**

Statistical analyzes were performed using the MedCalc Statistical Software version 18.2.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). Quantitative data were expressed as median and interquartile range (non-normal distribution), and qualitative data were characterized by frequency and percentage. Comparisons between groups were performed using the Chi-Square test, Fisher’s exact test, or Mann-Whitney test, whenever appropriate. To identify predictors of death, baseline characteristics were presented stratified by 30-day survival. For each marker we calculated the area under the curve using the trapezoidal method. This included all determinations of serum concentrations of biomarkers studied over time from the first measurement up to 12, 24, 48 or 72 hours. In order to find out how accurate a marker differentiates the deceased form the survivors, we used the area under the receiver operating characteristics (AUROC) curves. The cut-off value for each biomarker was calculated where specificity and sensibility were maximal. A predictive score for the mortality at 30 days was created with a logistic regression and it included the best performing markers. The score was calculated using the following formula: score = exp (c + b \cdot x) / 1 + exp(c + b \cdot x); where exp is the base of natural logarithms; c is the constant of the logistic regression; b is the coefficient of the predictor variable; x is value of the marker. A p value < 0.05 was considered statistically significant.

**Results**

Of the 152 consecutive patients resuscitated after CA and admitted to the ED, 40 were eligible and have been included in the study with a median age of 67 years (IQR: 59.2 to 76). In this study group 28 (70.0%) patients were men. Most frequent rhythms of CA were asystole and pulseless electrical activity, found in 28 cases (70.0%), with a median duration of resuscitation of 15 minutes (IQR: 7.75 to 28.75). Most patients developed post CA-shock after initial resuscitation [n = 29 (72.5%)] with a high mortality rate [n = 25 (86.2%)], especially due to post-CA shock [n = 18 (72.0%)] (Fig 1).

Cardiovascular disease was the main cause of CA with 29 (72.5%) cases and acute myocardial infarction (40.0%) was the most common presentation (all patients receiving coronary revascularization immediately after resuscitation) (Table 1).

Of the 40 enrolled patients, 12 (30.0%) survived and 28 (70.0%) died before 30 days: 6 patients (15.0%) died in the first 24 hours after admission but not within the first 6 hours, while other 6 patients (15.0%) died before reaching 48 hours of admission; 3 patients (7.5%) died between 48–72 hours and 13 patients (32.5%) died after 72 hours of admission but before completing 30 days of follow-up.

Cardiovascular comorbidities and higher disease severity scores were registered in non-survivors as otherwise expected. In survivors, the initial rhythm at presentation was ventricular fibrillation/pulseless ventricular tachycardia, with a shorter duration of resuscitation compared to those who died most often presented with asystole/pulseless electrical activity (Table 2).

**Biomarkers assay**

At each time interval, median serum levels of resistin and S-100B were significantly higher in non-survivors compared to survivors (p < 0.05). For NSE, plasma levels were significantly lower in survivors compared to non-survivors at 48 and 72 hours, respectively (p < 0.05) (Table 3).
Comparing serum concentrations of biomarkers at different time intervals, in non-survivors and survivors, shown that levels were significantly higher in non-survivors for resistin and S-100B (p < 0.05), with a relatively constant AUCs for resistin determinations (Table 4).

For predicting the mortality at 30 days, we created several predictive models using logistic regressions (Table 5). In each model we included 2 or 3 variables that achieved the highest AUC alone (Tables 3 and 4) in order to established which score could better predict the

Table 1. Causes of cardiac arrest and the causes of death at 30 days in relation to post-CA shock, neurological injury and sepsis.

| Causes of cardiac arrest | Non-survivors n = 28 (70.0%) |
|--------------------------|-------------------------------|
| Post-CA shock            | Neurological injury Sepsis    |
| Acute myocardial infarction 16 (40.0%) | 4 (14.80%) | 3 (10.71%) | 0 (0%) |
| Pulmonary Embolism 2 (5.0%) | 2 (7.14%) | 0 (0%) | 0 (0%) |
| Acute pulmonary edema 4 (10.0%) | 4 (14.28%) | 0 (0%) | 0 (0%) |
| Rhythm disorders related to heart disease 7 (17.5%) | 1 (3.57%) | 0 (0%) | 3 (10.71%) |
| Rhythm disorders related to electrolyte imbalances 2 (5.0%) | 2 (7.14%) | 0 (0%) | 0 (0%) |
| Acute respiratory failure 3 (7.5%) | 1 (3.57%) | 0 (0%) | 2 (7.14%) |
| Stroke 2 (5.0%) | 0 (0%) | 1 (3.57%) | 1 (3.57%) |
| Septic shock 4 (10.0%) | 4 (14.8%) | 0 (0%) | 0 (0%) |
mortality. The score was calculated using the equation described in materials and methods and the coefficients were provided by the regression.

After calculating the scores, we used the AUROC to determine which biomarker could differentiate more accurately the non-survivors from the survivors. The most accurate scores are

| Characteristics              | AUC          | Non-survivors (n = 28) | Survivors (n = 12) | p   |
|------------------------------|--------------|------------------------|--------------------|-----|
| Age, years, median (IQR)     | 0.735        | 69.0 (IQR:66.0 to 76.7) | 60.50 (IQR:45.0 to 66.7) | 0.02 |
| Sex, n (%)                   |              |                        |                    |     |
| Female                       | 9 (32.1)     | 3 (25.0)               |                    | 0.72 |
| Male                         | 19 (67.9)    | 9 (75.0)               |                    |     |
| Primary rhythm (%)           | VF/VT        | 2 (7.1)                | 10 (83.3)          | <0.001 |
|                              | Asystole/PEA | 26 (92.9)              | 2 (16.7)           |     |
| Duration of CPR, minutes, median (IQR) | 0.713    | 15.0 (IQR:10.0 to 30.0) | 6.0 (IQR:4.2 to 20.0) | 0.03 |
| Cardiovascular comorbidities, n (%) | 22 (78.6) | 4 (33.3)               |                    | 0.01 |
| Non-cardiovascular comorbidities, n (%) | 14 (50.0) | 4 (33.3)               |                    | 0.49 |
| SOFA score at admission, median (IQR) | 0.815 | 15.0 (IQR:13.0 to 17.0) | 11.0 (IQR:6.2 to 13.0) | 0.002 |
| TH, n (%)                    | 22 (81)      | 5 (18.5)               |                    | 0.03 |
| Lactate at admission, median (IQR) | 0.890 | 11.3 (IQR: 10.1 to 13.3) | 5.7 (IQR: 2.8 to 9.1) | <0.001 |
| Creatinine at admission, median (IQR) | 0.734 | 1.4 (IQR: 1.1 to 1.9) | 1 (IQR: 0.9 to 1.3) | 0.008 |

PEA = pulseless electrical activity; VF = ventricular fibrillation, VT = ventricular tachycardia
CPR = cardiopulmonary resuscitation; IQR = interquartile range
SOFA = Sequential Organ Failure Assessment Score; Highest SOFA = the highest SOFA score recorded in the first 72 hours
TH = therapeutic hypothermia

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Table 3. Median serum levels and AUC of resistin, S-100B and NSE at admission and during the first 72 hours.

| Variable     | Time interval | AUC | Non-survivors (n = 28) | Survivors (n = 12) | p   |
|--------------|---------------|-----|------------------------|--------------------|-----|
| Resistin (ng/ml) | 0 hours       | 0.751 | 9.35 (IQR: 5.6 to 13.7) | 5.80 (IQR: 1.9 to 6.9) | 0.01 |
|              | 6 hours       | 0.807 | 11.80 (IQR: 7.1 to 19.8) | 4.30 (IQR: 3.2 to 9.3) | 0.002 |
|              | 12 hours      | 0.875 | 15.85 (IQR: 10.8 to 24.2) | 4.50 (IQR: 1.6 to 11.2) | <0.001 |
|              | 24 hours      | 0.795 | 16.50 (IQR: 10.2 to 26.5) | 6.75 (IQR: 3.6 to 9.6) | 0.001 |
|              | 48 hours      | 0.779 | 13.45 (IQR: 5.2 to 21.0) | 3.65 (IQR: 1.6 to 7.4) | 0.004 |
|              | 72 hours      | 0.763 | 11.00 (IQR: 6.2 to 17.8) | 5.35 (IQR: 2.5 to 8.0) | 0.02 |
| S-100B (pg/ml)  | 0 hours       | 0.798 | 52.80 (IQR: 15.7 to 122.8) | 5.95 (IQR: 3.7 to 20.9) | 0.003 |
|              | 6 hours       | 0.918 | 40.90 (IQR: 4.8 to 190.3) | 4.80 (IQR: 3.3 to 8.9) | <0.001 |
|              | 12 hours      | 0.839 | 79.90 (IQR: 9.5 to 165.2) | 4.60 (IQR: 3.3 to 12.8) | 0.001 |
|              | 24 hours      | 0.782 | 56.30 (IQR: 10.9 to 199.5) | 4.60 (IQR: 3.3 to 12.8) | 0.001 |
|              | 48 hours      | 0.776 | 31.50 (IQR: 11.6 to 201.5) | 6.60 (IQR: 3.6 to 15.7) | 0.009 |
|              | 72 hours      | 0.798 | 25.10 (IQR: 7.8 to 80.7) | 4.80 (IQR: 3.3 to 12.8) | 0.01 |
| NSE (ng/ml)   | 0 hours       | 0.509 | 7.30 (IQR: 3.27 to 15.87) | 8.05 (IQR: 2.10 to 18.15) | 0.92 |
|              | 6 hours       | 0.618 | 15.40 (IQR: 4.77 to 47.77) | 10.85 (IQR: 3.92 to 19.62) | 0.24 |
|              | 12 hours      | 0.652 | 20.05 (IQR: 6.52 to 80.77) | 12.10 (IQR: 1.77 to 20.12) | 0.13 |
|              | 24 hours      | 0.506 | 19.85 (IQR: 6.65 to 97.65) | 10.60 (IQR: 4.67 to 20.17) | 0.17 |
|              | 48 hours      | 0.779 | 72.20 (IQR: 9.70 to 211.87) | 5.40 (IQR: 1.35 to 11.70) | 0.006 |
|              | 72 hours      | 0.750 | 67.00 (IQR: 4.75 to 194.60) | 4.00 (IQR: 1.90 to 23.95) | 0.03 |

AUC = area under the curve; IQR = interquartile range

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presented in Table 6. We did not find statistically significant differences between any of the AUCs of the scores.

When we compared the predictive value of resistin alone at 12 hours with the combination of S-100B and resistin at 12 hours we found a $p = 0.07$. The sensitivity was almost the same (85.7%) but the specificity was lower for resistin (75% vs. 91.6%). When we compared the predictive value of resistin alone at 12 hours with the area for resistin and S-100B combined score between 0–12 hours we found a $p = 0.08$. When we compared the predictive value of resistin alone at 12 hours with the resistin, NSE and S-100B combined score at 12 hours we found a $p = 0.1$. When we compared the predictive value of resistin alone at 12 hours with area for

Table 4. Comparison of area for markers measurements between non-survivors and survivors.

| Variable               | AUC     | Non-survivors (n = 28) | Survivors (n = 12) | P       |
|------------------------|---------|------------------------|--------------------|---------|
| Area for resistin ng x h/ml |         |                        |                    |         |
| 0–12 hours (3 measurements) | 0.792   | 26.0 (IQR: 14.0–37.5)  | 9.5 (IQR: 4.2–22.5) | <0.001  |
| 0–24 hours (4 measurements) | 0.788   | 27.2 (IQR: 18.3–37.5)  | 14.9 (IQR: 8.3–21.8) | 0.001   |
| 0–48 hours (5 measurements) | 0.795   | 22.0 (IQR: 14.5–38.7)  | 12.7 (IQR: 5.3–15.9) | 0.003   |
| 0–72 hours (6 measurements) | 0.814   | 44.9 (IQR: 30.5–71.9)  | 23.4 (IQR: 15.4–33.7) | 0.008   |
| Area for S-100B pg x h/ml |         |                        |                    |         |
| 0–12 hours (3 measurements) | 0.833   | 75.0 (IQR: 24.5–233.0) | 12.0 (IQR: 7.2–30.5) | <0.001  |
| 0–24 hours (4 measurements) | 0.647   | 64.8 (IQR: 15.5–129.3) | 23.2 (IQR: 12.9–30.2) | 0.011   |
| 0–48 hours (5 measurements) | 0.692   | 55.1 (IQR: 22.6–251.8) | 26.4 (IQR: 15.1–31.1) | 0.041   |
| 0–72 hours (6 measurements) | 0.750   | 122.5 (IQR: 38.0–372.0) | 37.8 (IQR: 22.5–73.8) | 0.034   |
| Area for NSE ng x h/ml |         |                        |                    |         |
| 0–12 hours (3 measurements) | 0.455   | 19.0 (IQR: 9.0–61.5)   | 27.0 (IQR: 6.0–38.0) | 0.215   |
| 0–24 hours (4 measurements) | 0.551   | 39.8 (IQR: 11.2–47.5)  | 23.5 (IQR: 10.7–52.2) | 0.097   |
| 0–48 hours (5 measurements) | 0.763   | 123.0 (IQR: 28.5–313.5) | 19.0 (IQR: 6.25–37.5) | 0.008   |
| 0–72 hours (6 measurements) | 0.737   | 108.0 (IQR: 25.0–318.0) | 16.5 (IQR: 8.5–48.2) | 0.044   |

Table 5. Logistic regression for 30-days mortality.

| Variables                                      | B       | P     | OR  | 95% C.I. for OR Lower | Upper |
|------------------------------------------------|---------|-------|-----|-----------------------|-------|
| Model for resistin and S-100B combined score at 12 hours |         |       |     |                       |       |
| S-100B pg/ml at 12 hours                       | 0.058   | 0.1   | 1.060 | .975                   | 1.153 |
| Resistin ng/ml at 12 hours                     | 0.262   | 0.020 | 1.300 | 1.042                  | 1.621 |
| Constant                                       | -3.057  | 0.01  | 0.047 | –                     | –     |
| Model for resistin, NSE and S-100B combined score at 12 hours |         |       |     |                       |       |
| S-100B pg/ml at 12 hours                       | 0.136   | 0.2   | 1.146 | 0.899                  | 1.461 |
| Resistin ng/ml at 12 hours                     | 0.374   | 0.02  | 1.453 | 1.050                  | 2.010 |
| NSE ng/ml at 12 hours                          | -0.024  | 0.1   | 0.977 | 0.949                  | 1.006 |
| Constant                                       | -4.078  | 0.04  | 0.017 | –                     | –     |
| Model for area for resistin and S-100B combined score between 0–12 hours |         |       |     |                       |       |
| Area for resistin 0–12 hours                    | 0.135   | 0.02  | 1.145 | 1.019                  | 1.285 |
| Area for S-100B 0–12 hours                      | 0.033   | 0.1   | 1.034 | 0.993                  | 1.076 |
| Constant                                       | -3.423  | 0.01  | 0.033 | –                     | –     |
| Model for areas for resistin, NSE and S-100B combined score between 0–12 hours |         |       |     |                       |       |
| Area for resistin 0–12 hours                    | 0.204   | 0.02  | 1.226 | 1.026                  | 1.465 |
| Area for S-100B 0–12 hours                      | 0.055   | 0.1   | 1.056 | 0.983                  | 1.134 |
| Area for NSE 0–12 hours                         | -0.014  | 0.1   | 0.986 | 0.969                  | 1.003 |
| Constant                                       | -4.530  | 0.03  | 0.011 | –                     | –     |
| Model for resistin and S-100B at 6 hours and lactate at 0 hours combined score |         |       |     |                       |       |
| Lactate mmol/L at 0 hours                       | 1.880   | 0.2   | 6.553 | 0.206                  | 208.9 |
| S-100B pg/ml at 6 hours                         | 1.415   | 0.3   | 4.116 | 0.261                  | 64.824 |
| Resistin ng/ml at 6 hours                       | 1.269   | 0.2   | 3.559 | 0.493                  | 25.701 |
| Constant                                       | -37.919 | 0.2   | 0     | –                     | –     |
resistin, NSE and S-100B combined score between 0–12 hours we found a p = 0.1. When we compared the predictive value of resistin alone at 12 hours with resistin and S-100B at 6 hours and lactate at 0 hours combined score we found a p = 0.03.

We examined the usefulness of the markers for predicting the mortality at 24 hours, since guidelines usually cannot indicate the predictors for such an early event. Only high resistin values had a statistically significant association with the 24-hours morality: at 0 hours (p = 0.008), at 6 hours (p = 0.02) and at 12 hours (p = 0.03).

Considering that at this point the NSE is useful for predicting mortality, if is measured in dynamics and after 48–72 hours, we compared it with the score that combined resistin and S100 at 12 hours (Fig 2). We obtained an AUC of 0.911 (0.742 to 0.985) for the score and an AUC of 0.810 (0.618 to 0.932) for NSE alone. The difference between AUCs was not statistically significant (p = 0.2).

### Table 6. AUCs for the predictive scores.

| Predictive score                                      | AUC (CI95%)     | Cut-off | Sensitivity (CI 95%) | Specificity (CI 95%) | p          |
|-------------------------------------------------------|-----------------|---------|----------------------|----------------------|------------|
| Resistin and S100B combined score at 12 hours         | 0.938 (0.813–0.989) | > 0.65  | 85.71 (67.3–96.0)    | 91.67 (61.5–99.8)    | <0.001     |
| Resistin, NSE and S100B combined score at 12 hours    | 0.955 (0.839–0.995) | > 0.73  | 82.14 (63.1–93.9)    | 100.00 (73.5–100.0)  | <0.001     |
| Area for resistin and S100B combined score between 0–12 hours | 0.937 (0.813–0.989) | > 0.58  | 82.14 (63.1–93.9)    | 91.67 (61.5–99.8)    | <0.001     |
| Area for resistin, NSE and S100B combined score between 0–12 hours | 0.958 (0.843–0.996) | > 0.69  | 85.71 (67.3–96.0)    | 100.00 (73.5–100.0)  | <0.001     |
| Resistin and S100B at 6 hours and lactate at 0 hours combined score | 0.994 (0.901–1)   | > 0.57  | 96.4 (81.7–99.9)     | 100 (73.5–100.0)     | <0.001     |

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Discussions

In the last few years it has been shown that resistin levels are well correlated with prognosis of critically ill patients [18]. This correlation may be attributed to the fact that resistin is involved in the release of increased levels of pro-inflammatory biomarkers—IL-6, IL-12 and TNF-α—that are involved in organ failure caused by sepsis [14, 16].

Taking into consideration that an inflammatory process is involved in hypoxic-ischemic organ injury after CA, we investigated a possible correlation between resistin serum levels and the outcome in resuscitated patients and possible predictive value of resistin combined with S-100B and NSE. To our knowledge this is the first study to evaluate resistin as a biomarker for the outcome of resuscitated patients.

Previous studies have focused on the role of NSE and S-100B protein [3, 22] in predicting outcome in patients resuscitated after CA, in order to direct maximal resources for the treatment of these patients [4–6, 9–12], although the time interval for markers assay seems to be crucial in defining their role for predicting patients’ outcome [3, 5]. Today these markers are included in resuscitation guidelines.

Stammet P et al., found that NSE levels were well correlated with the outcome in resuscitated patients after CA, as an indicator of increased risk of death and poor neurologic outcome [5]. Similarly, Wiberg S et al. showed that NSE is a strong marker for predicting mortality at 48 hours after resuscitation, in patients with initially successfully resuscitated CA although a cut-off value with 100% specificity (false positive rate of 0) and high sensitivity could not yet be determined [4, 6].

Our study found similar results, showing that NSE levels were significantly elevated in non-survivors, with the highest sensitivity and specificity for predicting mortality if measured at 48 hours from the event.

Wiberg S. et al showed that S-100B is an important marker in predicting mortality after successfully resuscitated CA [6], although it’s role in resuscitation is not fully attributable. Similar results are reported by us in this study. We showed that S100B levels were significantly higher in patients who died, at all-time intervals, making S100B a highly predictive and more accurate biomarker for 30-day mortality than NSE.

For resistin, our results showed that levels (including median serum concentrations levels) were significantly increased at each time interval of assay in non-survivors compared to survivors with relatively constant AUCs and high predictive value for 30-day mortality compared with NSE.

Starting from these results, for predicting the mortality at 30 days, we created several models following the pattern that could better differentiate the non-survivors from the survivors. Taking into account the cost-effective ratio (the number of determinations over time, the current cost of biomarker kits), we could demonstrate that six biomarkers determinations that combined resistin with S100 in the first 12 hours is sufficient for early prediction of outcome in patients who survived after a CA (deceased versus survivor).

We must agree and point out, that both biomarkers had a lower sensitivity than specificity for all determinations (although they are very close). This reinforces the suggestion that interpreting the values of these biomarkers in relation to post-cardiac arrest survival should be part of a multifactorial approach of the clinician.

One of the biological variables of interest in determining the prognosis of critically ill patients is serum lactate. Recent studies demonstrate that elevated serum lactate levels determined upon patient arrival in the emergency department is associated with unfavorable prognosis of patients who presented an episode of CA [24, 25]. The results were promising and from the many combinations of biomarkers lactate value at arrival and serum resistin and
S100B values at 6 hours best predict mortality (AUC = 0.994, Sp = 100%, Se = 96.4%, p < 0.001). Although we are tempted to believe that we have found the ideal predictive combination, the fairly wide confidence interval of the regression variables on which we built the score impose the need for a larger group of patients to increase the accuracy of the model.

Regarding the usefulness of biomarkers in the prediction of 24-hour early mortality in a patient who initially survived a successfully resuscitated CA, resistin was the only one that was statistically significantly associated with morality.

All of these findings support the idea that resistin may be an important future biomarker in predicting early prognosis after resuscitation, due to its role in the inflammatory processes underlying the hypoxic-ischemic organ injuries after a CA. By combining resistin with S-100B and with S-100B and lactate, predictive value of these biomarkers increased.

Our study is limited by the inclusion of a small number of resuscitated patients, comparable however with other similar studies. At the same time, we could not validate the predictive score due to the difficulty in recruiting patients. We mention that all the study patients were treated with conventional CPR and the results of the study could not extrapolate to patients treated with ECPR.

**Conclusion**

In our study, serum levels of resistin alone or a combination of resistin with S-100B or resistin with S-100B and lactate were highly predictive of 30 days mortality in resuscitated patients after CA. Further studies on larger number of patients are needed to confirm our hypothesis.

**Supporting information**

S1 Database.

(XLSX)

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References

1. Scolletta S, Donadello K, Santonocito C, Franchi F, Taccone FS. Biomarkers as predictors of outcome after cardiac arrest. Expert Rev Clin Pharmacol. 2012; 5(6):687–99. https://doi.org/10.1586/ecp.12.64 PMID: 23234326

2. Einav S, Kaufman N, Algr N, Strauss-Livianan N, Kark JD. Brain biomarkers and management of uncertainty in predicting outcome of cardiopulmonary resuscitation: a nomogram paints a thousand words. Resuscitation. 2013; 84(8):1083–8. https://doi.org/10.1016/j.resuscitation.2013.01.031 PMID: 23391666

3. Nolan JP, Soar J, Cariou A, Cronberg T, Moulaert VR, Deakin CD, et al. European Resuscitation Council and European Society of Intensive Care Medicine Guidelines for Post-resuscitation Care 2015: Section 5 of the European Resuscitation Council Guidelines for Resuscitation 2015. Resuscitation. 2015; 95:202–22. https://doi.org/10.1016/j.resuscitation.2015.07.018 PMID: 26477702

4. Wiberg S, Hassager C, Stammet P, Winther-Jenssen M, Thomsen JH, Erlinge D, et al. Single versus Serial Measurements of Neuron-Specific Enolase and Prediction of Poor Neurological Outcome in Persistently Unconscious Patients after Out-Of-Hospital Cardiac Arrest—A TTM-Trial Substudy. PLoS One. 2017; 12(1):e0168894. https://doi.org/10.1371/journal.pone.0168894 PMID: 28099439

5. Nolan JP, Soar J, Cariou A, Cronberg T, Moulaert VR, Deakin CD, et al. European Resuscitation Council and European Society of Intensive Care Medicine Guidelines for Post-resuscitation Care 2015: Section 5 of the European Resuscitation Council Guidelines for Resuscitation 2015. Resuscitation. 2015; 95:202–22. https://doi.org/10.1016/j.resuscitation.2015.07.018 PMID: 26477702

6. Wiberg S, Kjaergaard J, Kjaergaard B, Møller B, Sørensen AM, et al. Neuron-Specific Enolase as a Predictor of Death or Poor Neurological Outcome After Out-of-Hospital Cardiac Arrest and Targeted Temperature Management at 33 degrees C and 36 degrees C. J Am Coll Cardiol. 2015; 65(19):2104–14. PMID: 25975474

7. Langeland H, Bergum D, Loberg M, Bjørnstad K, Damøs JK, Mollnes TE, et al. Transitions Between Circulatory States After Out-of-Hospital Cardiac Arrest: Protocol for an Observational, Prospective Cohort Study. JMIR Res Protoc. 2018; 7(1):e17. https://doi.org/10.2196/resprot.8558 PMID: 29551497

8. Elmer J, Jeong K, Abebe KZ, Guyette FX, Murugan R, Callaway C, et al. Serum Neutrophil Gelatinase-Associated Lipocalin Predicts Survival After Resuscitation From Cardiac Arrest. Crit Care Med. 2016; 44(1):111–9. https://doi.org/10.1097/CCM.0000000000001357 PMID: 26457752

9. Einav S, Kaufman N, Algr N, Kark JD. Modeling serum biomarkers S100 beta and neuron-specific enolase as predictors of outcome after out-of-hospital cardiac arrest: an aid to clinical decision making. J Am Coll Cardiol. 2012; 60(4):304–11. https://doi.org/10.1016/j.jacc.2012.04.020 PMID: 22813607

10. Calderon LM, Guyette FX, Doshi AA, Callaway CW, Rittenberger JC. Combining NSE and S100B with clinical examination findings to predict survival after resuscitation from cardiac arrest. Resuscitation. 2014; 85(8):1025–9. https://doi.org/10.1016/j.resuscitation.2014.04.020 PMID: 24795283

11. Daubin C, Quentin C, Allouche S, Etard O, Gaillard C, Seguin A, et al. Serum neuron-specific enolase as predictor of outcome in comatose cardiac-arrest survivors: a prospective cohort study. BMC Cardiovasc Disord. 2011; 11:48. https://doi.org/10.1186/1471-2261-11-48 PMID: 21824428

12. Stammet P, Wagner DR, Gilson G, Devaux Y. Modeling serum level of s100beta and bispectral index to predict outcome after cardiac arrest. J Am Coll Cardiol. 2013; 62(9):851–8. PMID: 23684684

13. Sun BD, Liu HM, Nie SN. S100B protein in serum is elevated after global cerebral ischemic injury. World J Emerg Med. 2013; 4(3):165–8.PMID: 25215112

14. Fain JN, Cheema PS, Bahouth SW, Lloyd Hiler M. Resistin release by human adipose tissue explants in primary culture. Biochem Biophys Res Commun. 2003; 300(3):674–8. PMID: 12507502

15. Patel L, Buckels AC, Kinghorn IJ, Murdoch PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun. 2003; 300(2):472–6. PMID: 12504108
16. Macdonald SP, Stone SF, Neil CL, van Eeden PE, Fatovich DM, Arendts G, et al. Sustained elevation of resistin, NGAL and IL-8 are associated with severe sepsis/septic shock in the emergency department. PLoS One. 2014; 9(10):e110678. https://doi.org/10.1371/journal.pone.0110678 PMID: 25343379

17. Chen C, Jiang J, Lu JM, Chai H, Wang X, Lin PH, et al. Resistin decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. Am J Physiol Heart Circ Physiol. 2010; 299(1):H193–201. https://doi.org/10.1152/ajpheart.00431.2009 PMID: 20435848

18. Koch A, Gressner OA, Sanson E, Tacke F, Trautwein C. Serum resistin levels in critically ill patients are associated with inflammation, organ dysfunction and metabolism and may predict survival of non-septic patients. Crit Care. 2009; 13(3):R95. https://doi.org/10.1186/cc7925 PMID: 19545363

19. Jamaluddin MS, Weakley SM, Yao Q, Chen C. Resistin: functional roles and therapeutic considerations for cardiovascular disease. Br J Pharmacol. 2012; 165(3):622–32. https://doi.org/10.1111/j.1476-5381.2011.01369.x PMID: 21545576

20. Perkins GD, Handley AJ, Koster RW, Castreñ M, Smyth MA, Olasveengen T, et al. European Resuscitation Council Guidelines for Resuscitation 2015: Section 2. Adult basic life support and automated external defibrillation. Resuscitation. 2015; 95:81–99. PMID: 26477420

21. Soar J, Nolan JP, Böttiger BW, Perkins GD, Lott C, Carli P, et al. European Resuscitation Council Guidelines for Resuscitation 2015: Section 3. Adult advanced life support. Resuscitation. 2015; 95:100–47. https://doi.org/10.1016/j.resuscitation.2015.07.016 PMID: 26477701

22. Callaway CW, Soar J, Aibiki M, Böttiger BW, Brooks SC, Deakin CD, et al. Part 4: Advanced Life Support: 2015 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Circulation. 2015; 132(16 Suppl 1):S84–145. https://doi.org/10.1161/CIR.0000000000000273 PMID: 26472860

23. Vincent JL, Moreno R, Takala J, Williats S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive Care Med. 1996; 22(7):707–10. PMID: 8844239

24. Donnino MW, Andersen LW, Giberson T, Gajeski DF, Abella BS, Peberdy MA, et al. Initial lactate and lactate change in post-cardiac arrest: a multicenter validation study. Crit Care Med. 2014; 42(8):1804–11. https://doi.org/10.1097/CCM.0000000000000332 PMID: 24776506

25. Park YJ, Kim DH, Kim SC, Kim TY, Kang C, Lee SH, et al. Serum lactate upon emergency department arrival as a predictor of 30-day in-hospital mortality in an unselected population. PLoS One. 2018; 13(1):e0190519. https://doi.org/10.1371/journal.pone.0190519 PMID: 29293610