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The Journal of Critical Care Medicine 2019;5(3):97-102

RESEARCH ARTICLE

DOI: 10.2478/jccm-2019-0018

Determination of Cut-off Serum Values for Resistin and S100B Protein in Patients Who Survived a Cardiac Arrest

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Abstract

Introduction: In an attempt to identify patients who have successfully survived a resuscitated cardiac arrest (CA), attention is drawn to resistin and S100B protein, two biomarkers that have been studied in relation to CA.

Aim: The study aimed to identify the potential cut-off serum values for resistin and S100B in patients who had CA, compared to healthy volunteers, given that, currently, none of the markers have normal and pathological reference range limits for human assay levels related to this pathology.

Materials and Methods: Forty patients, resuscitated after out-of-hospital CA and forty healthy controls, were included in the study. All patients were followed up for seventy-two hours after CA or until death. Blood samples for biomarkers were collected on admission to the ED (0-time interval) and at 6, 12, 24, 48 and 72 hours following resuscitation. Only one blood sample was collected from the controls. The serum concentrations of biomarkers were measured.

Results: For each time interval, median serum levels of resistin and S100 B were significantly higher in patients with CA compared to healthy controls. The cut-off value for resistin in patients with CA, at the 12-hours versus controls, was > 8.2 ng/ml. The cut-off value for S100B in patients with CA versus controls recorded at 6 hours, was > 11.6 pg/ml.

Conclusion: Serum levels of resistin and S100B are higher among resuscitated CA patients compared to controls.

Keywords: resistin, S100B protein, biomarkers, cardiac arrest

Received: 18 May 2019 / Accepted: 30 July 2019

Introduction

Despite fifty years of research, cardiac arrest (CA) and complications resulting from hypoxic organ injury, remains one of the most significant challenges faced by physicians, knowing that the vast majority of patients with CA have an unfavourable prognosis with a higher chance of death and severe neurological disabilities [1-3]. In the last few years, efforts have been focused on the best way to detect those patients who have a chance to survive and to recover.

In an attempt to identify patients who survived after resuscitated CA, attention is drawn to resistin and S100B protein, two biomarkers that have been studied relatively recently in relation to CA [4-8].

Resistin, a protein from the family of resistin-like molecules, expressed in humans by adipocytes tissue cells, peripheral blood mononuclear cells, macrophages and bone marrow cells, is a biomarker of inflammation that can predict mortality in close correlation with the severity of organ dysfunction in patients with critical illness such as sepsis and cardiac arrest [4, 9-13].

S100B is a structural protein of the S-100 protein family, released from brain and myocardium tissue as well as from adipocytes. In recent years, elevated serum levels of this protein in patients with CA have been

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associated with both mortality and severe brain injury [1, 2, 4, 6, 7, 14, 15].

The implementation of resuscitation manoeuvres and the elapsed time until the resumption of spontaneous circulation may establish a post-CA syndrome [2]. The components of the post-CA syndrome, like myocardial injury and the persistence of the pathology that induced the CA, are factors that are linked to the prognosis of patients in post-resuscitation. Brain injury and associated impaired cerebrovascular autoregulation, cerebral oedema, post-ischemic neurodegeneration and post-CA shock are two other major entities that dramatically increase the morbidity and mortality of patients who have survived a CA.

Post-CA shock is based on ischemia-reperfusion injury and exacerbated activation of systemic inflammatory response mechanisms [2, 3]. This shock may lead to multiple organ failure and increase the chance of premature mortality [16].

Since both resistin and S100B have been studied as prognostic markers in relation to CA, the aim of the study was to identify the potential cut-off serum values in patients who had CA compared to healthy controls, taking into consideration that currently none of the markers have described normal and pathological reference intervals limits for human assay levels related to this pathology.

Materials and Methods

A prospective, analytical, longitudinal, observational, cohort study included consecutive patients, admitted to the ED of Cluj-Napoca County Emergency Clinical Hospital between May 2016 and October 2017, who had an out-of-hospital CA and had been successfully resuscitated.

The Ethics Committee approved the study of "Iuliu Hatieganu" University of Medicine and Pharmacy, registration number 59/14.03.2016.

Patients were included following informed proxy consent.

Inclusion criteria were that patients were aged between 18 – 85 years and had been successful resuscitation after OHCA.

Exclusion criteria included patients who were aged under 18 and over 85 years at the time of admission to hospital, pregnancy, CA due to trauma, acute bleeding from non-traumatic conditions, hypothermia, terminal neoplastic disease, re-arrest with unsuccessful resuscitation within six hours after arrival at the hospital, inmates and lack of informed consent.

The management of the patients in the ED and the definition of post-CA shock were previously described according to international guidelines [4].

Healthy controls were selected from the medical staff, with no reported illness according to the annual occupational medical examination report, and after obtaining informed consent. The age of volunteers ranged from 18 to 55 years.

Laboratory assays

Blood samples were collected on admission to the ED (0-time interval), and at 6, 12, 24, 48 and 72 hours following resuscitation, using a 5 ml serum separator and clot activator tubes. Haemolysed samples were excluded, and new blood samples were immediately repeated. Samples were centrifuged at 3000 RPM during the first 60 minutes after collection and were stored at -70°C. According to the manufacturer's instructions, serum concentrations of resistin and S100B were analysed using a quantitative sandwich immunoassay technique (ELISA; BioVendor, LM, Czech Republic).

All patients were followed up for seventy-two hours after being included in the study or until death.

For healthy controls, a single blood sample was collected, followed by the same protocol regarding centrifugation, preservation and processing of samples as previously described for CA patients [4].

Statistical analysis

Statistical analysis was performed using MedCalc Statistical Software version 18.2.1 (Med Calc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018).

Quantitative data were expressed as the median and interquartile range (non-normally distributed variables), and qualitative data were expressed as frequency and percentages. Comparisons between groups were performed using the Mann-Whitney test.

The mean difference for resistin at 0 hours was 4.26 ng/ml between patients and controls. For a type 1 (alpha) error of 0.05 and a type 2 (beta) error of 0.01, we determined a sample size of 31 subjects per group. The power of the study was calculated as 99%.

The Area under the ROC Curve (AUROC) and Area under Curve (AUC) values were used to calculate the

cut-off value for biomarkers. The cut-off value for each biomarker was calculated, where specificity and sensitivity were maximal. The significance level alpha was 0.05.

Results

The study included forty patients successful resuscitated after CA [Group A] and forty healthy controls [Group B]. Both groups met the stated inclusion criteria. The median age was 67 years (IQR: 59.2 to 76.0) in Group A and 34 years (IQR: 27.0 to 40.0) in Group B. Of patients in Group A, 12 (30%) were women. In Group B, there was gender equality.

The most recorded rhythms of CA in Group A were asystole [n = 23 (57.5%)] and pulseless electrical activity [n = 3 (12.5%)].

In Group A, 15 (37.5%) died in the first three days, six of these in the first 24 hours, six within 48 hours, and three between 48 hours and seventy-two hours. Thirteen more (32.5%) died between 72 hours and thirty days.

Cardiovascular disease was the primary cause of CA in 31 (77.5%) patients. Of all the patients with CA included in the study, 29 (72.5%) developed post-CA shock immediately after resuscitation. Of them, only 4 (13.79%) survived up to 30 days.

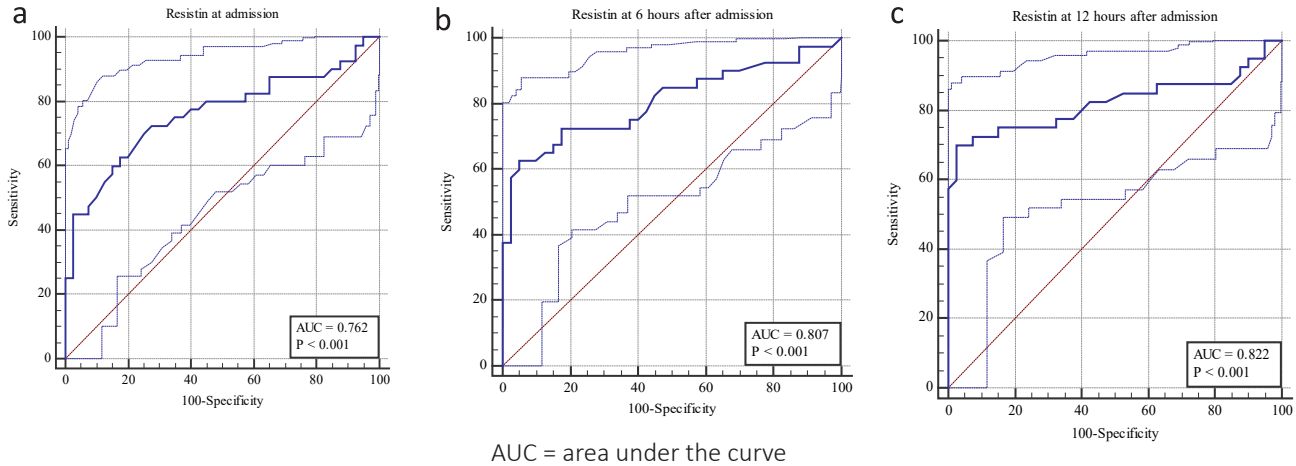
Median serum levels of resistin and S100B recorded in Group A at any time interval were higher than those recorded in Group B. At 12 hours, when the first deaths were registered, the median serum levels of resistin and S100B in Group A were three times higher than in controls (**Table 1**).

The AUC values for resistin for the first 3 measurements, when comparing Group A with Group B, shows that the best AUC value is obtained for the first 12 hours (**Figure 1**).

Table 1. Serum levels of biomarkers in the first three days, a comparison between Group A and Group B

Parameters	Time intervals (hours)	Group A*	Group B *	p -values
Resistin ng/ml	0	7.15 (4.67 to 11.85)	4.10 (2.50 to 5.75)	< 0.001
	6	9.85 (4.47 to 17.75)		< 0.001
	12	13.55 (5.55 to 21.02)		< 0.001
	24	12.30 (6.77 to 21.00)		< 0.001
	48	7.25 (3.57 to 14.65)		0.004
	72	7.40 (3.6 to 11.90)		0.002
S100B pg/ml	0	25.85 (7.07 to 102.52)	5.45 (4.00 to 8.47)	< 0.001
	6	17.45 (5.60 to 137.10)		< 0.001
	12	19.70 (4.25 to 127.02)		0.001
	24	15.20 (5.60 to 134.62)		< 0.001
	48	14.10 (6.10 to 79.65)		0.001
	72	8.90 (3.80 to 36.55)		0.05

*Levels expressed as median (IOR= interquartile range)



AUC = area under the curve

Fig. 1. Comparison of AUC values for resistin for the first 3 measurements (Group A versus Group B)

Considering the highest resistin-related AUC values recorded in the first 12 hours, the cut-off value registered in Group A, versus with Group B was > 8.2 ng/ml, with a specificity of 97.5% and a related sensitivity of 70.0% (**Table 2**).

For S100B, the AUC values for the first 3 determinations for Group A with Group B are shown in **Figure 2**.

The cut-off value for S100B with the highest specificity and highest sensitivity was registered within the first six hours from admission (**Table 3**) with no statistical difference between the six hour and twelve hour determinations.

Discussion

Along with the clinical and imagistic criteria, biomarkers are most commonly used in the quantification and prognosis of organ lesions in critically ill patients. Moreover, the study of biomarkers plays an important role in current research when it comes to patients successful resuscitated after CA.

Recently, both S100B and resistin are important prognostic markers in post-CA patients. Increased serum levels of these biomarkers adequately correlated with patient mortality and the severity of organ injury [3, 4, 6-8, 13, 14, 19]. However, none of these biomarkers had normal values described by all the manufacturers, but only reference values. Neither was there a comparison between the pathological values registered in patients with CA and normal baseline values.

Following a literature review, this is the first study that assessed the cut-off serum values for resistin and S100B, making a comparison between healthy controls and successful patients resuscitated after CA.

Table 2. Cut-off values for resistin for the first 3 measurements following inclusion to the study

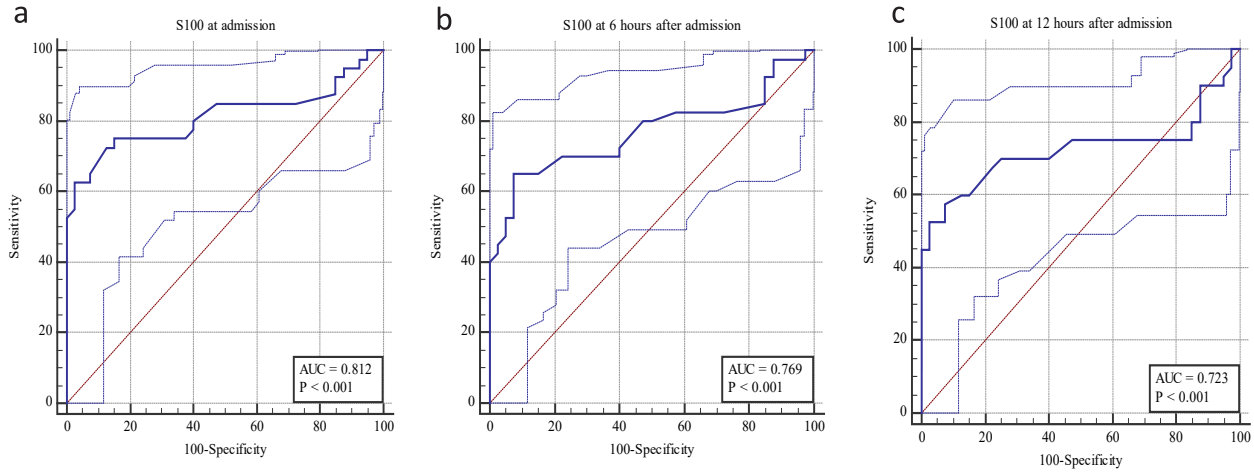
Resistin	AUC (CI95%)	Cut-off ng/ml	Sensitivity (CI 95%)	Specificity (CI 95%)	p
0 hours	0.762 (0.653 to 0.850)	> 5.5	72.50 (56.1 – 84.5)	72.50 (56.1 – 84.5)	<0.001
6 hours	0.807 (0.703 to 0.887)	> 7.6	62.50 (45.8 – 77.3)	95.00 (83.1 – 99.4)	<0.001
12 hours	0.822 (0.720 to 0.898)	> 8.2	70.00 (53.5 – 83.4)	97.50 (86.8 – 99.9)	<0.001

AUC = area under the curve

Table 3. Cut-off values for S100B for the first 3 measurements following inclusion to the study

S100B	AUC (CI95%)	Cut-off pg/ml	Sensitivity (CI 95%)	Specificity (CI 95%)	p
0 hours	0.812 (0.709 to 0.891)	> 8.9	75.00 (58.8- 87.3)	85.00 (70.2- 94.3)	<0.001
6 hours	0.769 (0.661 to 0.856)	> 11.6	65.00 (48.3- 79.4)	92.50 (79.6- 98.4)	<0.001
12 hours	0.723 (0.612 to 0.818)	> 11.6	57.50 (40.9- 73.0)	92.50 (79.6- 98.4)	<0.001

AUC = area under the curve



AUC = area under the curve

Fig. 2. Comparison of AUC values for S100B for the first 3 measurements (Group A versus Group B)

Koch A. et al. (2009) demonstrated that critical patients admitted to intensive care units have significantly higher resistin serum values than healthy volunteers and that critical patients diagnosed with sepsis had higher baseline values than those with severe cardiovascular disease. Their data were comparable to those of the current study especially the those measured at twelve hours post-CA when resistin reaches peak levels of 13.55 ng/ml in patients with CA versus 4.10 ng/ml in the control group [13].

Regarding S100B, several reported studies indicate a high variability of the normal values as well as the pathological values of the protein S100B protein, values obtained using different analysis techniques [20-23].

For this reason, the serum values of the S100B obtained Group A versus Group B patients in the current study, cannot be realistically compared to the other reported data in the literature.

Serum resistin and S100B levels were increased at all time intervals of the assay in Group A versus Group B in the current study.

It can be hypothesized that, immediately after resuscitation, biomarkers serum concentrations started to increase in CA survivors, probably as a consequence of the inflammatory response which develops secondary to hypoxic-anoxic and reperfusion injury occurring after the resumption of spontaneous circulation.

Another hypothesis is that the increase of each biomarker may be dependent on its half-life. Thus at twelve hours, when the first deaths occurred, both biomarkers expressed their highest levels, significantly higher compared, both to admission values and those recorded in healthy volunteers.

All these findings support the idea that both biomarkers play an essential role in the mechanisms involved in the evolution of the medical condition of CA patients. It remains to be seen to which extent these values are influenced by the heterogeneity of laboratory measurement techniques, the source of marker discharge, blood kinetics from the first hours after resuscitation, and the type of therapy administered to patients who have suffered a CA.

The number of patients in the study was enough to determine highly significant differences for biomarkers, as the calculated power of the study was 99%. There was an age difference between patients and controls but resulted from the selection of healthy controls. The upper age for healthy volunteers limit was chosen knowing that older age may be a risk factor for the development of cardiovascular disease [17, 18].

Conclusion

The data found that patients with successfully resuscitated CA had significantly increased levels of resistin and S100B as compared to healthy volunteers.

The cut-off values for resistin and S100B in patients with CA were the highest at the twelve hours measurement, as compared to the controls.

Conflict of interest

None to declare.

Acknowledgments

The study was financed by "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, through the Doctoral Research Project-2015 [No. 7690/42/15.04.2016]. The sponsors had no role in the design of the study, data collection, analysis and interpretation, writing of the manuscript or decision to submit the manuscript for publication. There was no additional external funding received for this study.

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